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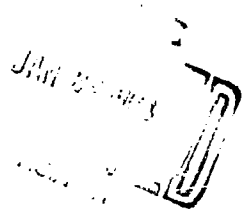
A STUDY OF THE MILITARY APPLICABILITY OF RESEARCH ON ASCORBIC ACID



Life Sciences Research Office
Federation of American Societies for Experimental Biology, Washington, D. C.

A STUDY OF THE MILITARY APPLICABILITY OF RESEARCH ON ASCORBIC ACID

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August 12, 1963

A Study of the Military Applicability of Research on Ascorbic Acid

FOREWORD

This study of the military applicability of research on ascorbic acid is based on the presentations and discussions of an ad hoc committee which was convened at the Headquarters of the Federation of American Societies for Experimental Biology on March 14 and 15, 1963 at the request of C. W. Clark, Major General, GS, Director of Army Research. The mission of the Committee is described in Appendix A and its membership in Appendix B. The text of the report and the recommendations have been approved by the Committee. Sincere appreciation is expressed to each member of the Committee for his interest and active participation.

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A Study of the Military Applicability of Research on Ascorbic Acid

GENERAL SUMMARY

The military importance of a better understanding of the requirements, functions, and mechanisms of action of ascorbic acid is highlighted by the fact that ascorbate is involved in some manner in the biogenesis of the protein, collagen, of connective tissue without which normal wound healing does not occur. The hydroxylation reaction, on which the synthesis of collagen's hydroxyproline depends, may be the metabolic reaction that underlies much of the vitamin's activity in the body. It is pertinent that hydroxylations are a part of the metabolism of tyrosine, of the formation of serotonin from tryptophan, and of the production of certain of the hormones of the adrenal medulla and cortex. These are reactions in which ascorbic acid has been tentatively implicated. The vitamin is also reported to exert a favorable influence on the absorption, transport, and utilization of iron; on the enzymes of the tricarboxylic acid cycle; on dentinogenesis by odontoblasts; on the formation of the mucopolysaccharides of connective tissues; and, on the folic acid-tetrahydrofolic acid system.

It is not possible to conclude from present evidence whether or not the ascorbate-dehydroascorbate mechanism plays an important role in electron transport reactions, although some such function may be associated with monodehydroascorbate as a labile intermediate form. There is urgent need of research that will clarify the distinction, if any, between ascorbate-dependent reactions that are primarily antiscorbutic in character and those in which an optimal physico-chemical environment is provided by the ascorbate oxidation-reduction system. Existence of reactions of the latter type would explain, in part, the nonspecificity that has been demonstrated in the case of isomers such as D-ascorbic acid, which possess partial activity. The failure to link ascorbic acid with a specific enzymatic reaction has been disconcerting. Nevertheless, man cannot survive without dietary ascorbic acid and the discovery of the cause of death in scurvy is a challenge meriting vigorous and skillful experimentation.

A beneficial effect of high levels of tissue saturation with ascorbic acid is believed to have been established in the case of healing processes, especially in skin grafts in patients with severe and extensive burns. In addition, a large body of evidence, based on animal studies, suggests that high tissue saturation may be advantageous to men exposed to severe environmental cold. The problem of the usefulness of high levels of tissue saturation in normal, sick, and injured adults exposed to stresses is complicated by factors concerning which insufficient knowledge is at hand. Among these factors are: the extent of unusual losses in the urine and of significant losses due to perspiration, hemorrhage, and serous exudates as a result of the stress; alteration of metabolism of ascorbate due to drugs, including chemicals that are occupational hazards and drugs used in therapy; alteration of the utilization of ascorbate arising from hormonal and other humoral factors; the specific effect of the stress in question or of multiple stresses; and, the surprisingly large individual variability in the relation of intake of ascorbic acid to its tissue concentration.

Ascorbic acid is non-toxic and it can be safely administered in relatively large amounts. However, the assumption is not justified that optimal efficiency of ascorbate metabolism over prolonged periods is compatible with unnecessarily high levels of tissue saturation with the vitamin. The validity of this conclusion awaits proof in the form of experimental evidence that explains the interrelationships of ascorbic acid and other nutrients and clarifies the effect of tissue saturation on metabolic processes.

It is noteworthy that it has not been possible to demonstrate unequivocally that there is superior nutritional health in normal adults who have blood ascorbate levels over 0.50 mg.% and tissue saturation levels over 50 to 60% compared with those who have blood levels near 0.20 mg.% and tissue saturation levels near 30%.

The rate of utilization of ascorbic acid can now be determined by analysis of expired air and urine following the administration of isotopically-labeled ascorbate. Extension of these studies to men undergoing various types of stress is highly desirable. It is particularly important to determine if, in fact, human tolerance to cold is increased by supplementary ascorbate, as appears to be the case in animals.

Stress, whether it is in the form of incapacitating physical exertion, infection, trauma, exposure to extremes of environmental temperature, low oxygen tension, or any other markedly abnormal circumstance is a constant hazard facing military personnel. Preventive measures that will assure the maximum possible protection against losses of operational efficiency are a necessity. Assurance of an adequate daily intake of ascorbic acid is such a preventive measure. The evidence supports the use of supplementary ascorbic acid in serious trauma and justifies its provisional use in cold stress. The value of supplementary ascorbate in other stresses remains to be determined.

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MILITARY APPLICABILITY OF RESEARCH ON ASCORBIC ACID

I. The Problem

Ascorbic acid has been identified as the antiscorbutic vitamin, the chemical compound required for the prevention and cure of the nutritional deficiency disease, scurvy. There is now no doubt regarding the indispensable character of this natural component of many fresh fruits and vegetables, the absence of which from the diet has been associated historically with weakness, hemorrhage, and death. Paradoxically, the exact details of the involvement of ascorbic acid in the metabolic reactions of the body have remained obscure although numerous hypotheses have been proposed to explain its important role. Among the investigations which have demonstrated, but not explained, its antiscorbutic efficacy there are findings which suggest that the ingestion of larger than scurvy-preventing amounts of the vitamin are advantageous for the maintenance of health and, especially, for the healing of injured tissues.

The problems, therefore, are (a) the evaluation of the evidence for benefits from the use of supplementary ascorbic acid by the soldier under stress and (b) the delineation of areas of additional research required to provide a definite answer to the problem if the evidence at any point is believed equivocal.

II. Present Knowledge of Ascorbic Acid

A. Ascorbic Acid.

The existence of an antiscorbutic factor was first demonstrated by Dr. James Lind, a Scottish physician, who described the efficacy of lemon juice in the treatment of scorbutic sailors in a "Treatise on Scurvy" published in 1753. A chemical compound, later to be shown to be identical with the vitamin, was isolated from adrenal tissue and named "hexuronic acid" in 1928 by A. Szent-Gyorgyi. The vitamin was subsequently isolated from lemon juice and identified as the antiscorbutic factor in 1932 by C. G. King and his co-workers. It is presently available in quantity as an inexpensive, synthetic product.

Ascorbic acid is readily soluble in water. Aqueous solutions containing less than one mg. per ml. decrease in antiscorbutic activity on exposure to air but stronger solutions are stable for several weeks. Although acidic solutions are relatively stable, even at high temperatures, ascorbic acid is inactivated by oxidation in neutral and alkaline solutions, especially if traces of copper are present. These properties account for the preservation of the antiscorbutic potency of canned tomato and citrus fruit juices, for the partial destruction of the vitamin during pasteurization of milk, and for its partial or complete loss during the cooking or dehydration of many foodstuffs. In certain instances these latter findings are based on the demonstrated loss of reducing activity and an inferred disappearance of antiscorbutic potency.

Ascorbic acid is reversibly converted to dehydroascorbic acid by mild oxidation. Both forms are antiscorbutic. A mechanism is present in tissues other than blood for the reduction of dehydroascorbic acid, possibly by the action of reduced glutathione and other sulfhydryl compounds. Hence, the vitamin is present in tissues almost wholly in the reduced form[1, 2]. Dehydroascorbic acid diffuses more rapidly across cell walls than ascorbic acid, probably because it is nonionic and more

soluble in lipid solvents. Inactive metabolic products which are excreted in the urine include diketogulonic acid and oxalic acid. Reports are contradictory regarding the oxidation of carbons of ascorbic acid to carbon dioxide in man and the detection of $C^{14}O_2$ in the expired air after the administration of a solution of ascorbic-1-C^{14} acid[3] may possibly be explained by degradative changes in the solution[4].

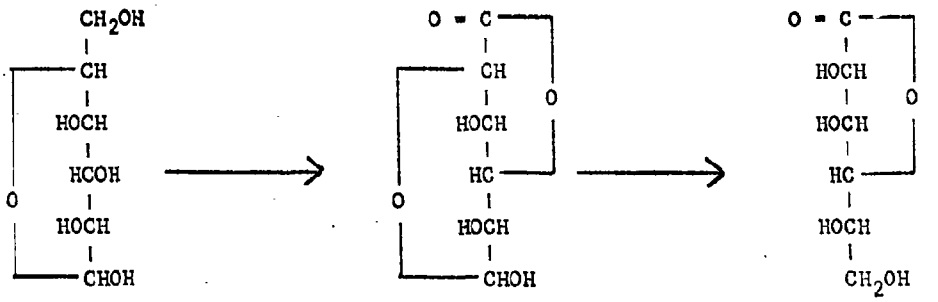
A rapid method for the estimation of ascorbic acid in the presence of dehydroascorbic acid is based on the reduction of the dye, 2-6-dichlorophenolindophenol by ascorbic acid. Dehydroascorbic acid is determined by measurement of the red color of the derivative formed by coupling dehydroascorbic acid with 2, 4-dinitrophenylhydrazine. Total ascorbate is measured by the same procedure after preliminary oxidation of ascorbic acid to dehydroascorbic acid[5].

Ascorbic acid is important in both plants and animals. It is synthesized in most mammalian species and the synthetic pathways for its biogenesis from glucose and galactose are known. The absence of one liver enzyme, L-gulono-γ-lactone oxidase, is the genetic defect in man, monkeys, and guinea pigs which accounts for the occurrence of scurvy in these species. Recently, the Indian fruit bat and the red-vented bulbul have been added to this unique and unfortunate group of scurvy-susceptible animals. The guinea pig has served as the experimental animal in most laboratory studies of the effects of a dietary deficiency of ascorbic acid. Results have agreed well with data obtained from the study of human cases of scurvy.

B. Scurvy.

Historical records of arctic expeditions, long sea voyages, periods of food shortages during military operations, and famine in settled communities are replete with descriptions of scurvy in man. A recent report testifies to the continuing threat of scurvy in infants as a result of faulty food practices[6]. Sore and spongy gums, abnormal development of teeth and skeleton, loosened teeth, swollen and tender joints, edema, anemia, anorexia, impaired wound healing, hemorrhage due to capillary fragility, and death characterize the scorbutic state in animals and in man. These effects have been extensively studied in experiments on guinea pigs, less so in experiments on primates and man. The findings have demonstrated conclusively the efficacy of vitamin C, ascorbic acid, as a preventive and curative antiscorbutic nutrient. Its function is multiple and includes a poorly understood role in oxidative metabolism and a regulatory role in the formation of intercellular material or "cement" by mesenchymal cells. The latter role involves the biogenesis of collagen of all fibrous tissue including bone, dentine, cartilage, and vascular epithelium.

The guinea pig on a normal diet of fresh grass ingests approximately 20 mg. of ascorbic acid daily. On diets lacking the vitamin, fatal scurvy appears after 3 to 4 weeks. Except in susceptible animals, 0.5 to 1.0 mg. daily will permit suboptimal growth and will prevent external evidences of scurvy. However, on intakes in this range plasma levels are low and internal evidences of scurvy appear. Hemorrhage in the knee joints occurs if plasma levels fall below 0.15 mg.% and abnormalities in the dental structures occur if the plasma levels are below 0.2 mg.%. An abnormal type of calcification is evident in the predentine layer, dentine, and pulp cavity. In addition, a characteristic disorganization and degeneration is observed in the odontoblasts, the cells most sensitive to ascorbic acid deficiency[7]. The possibility of synergism with other vitamins cannot be excluded and a deficiency of ascorbic acid may bring out other latent deficiencies.



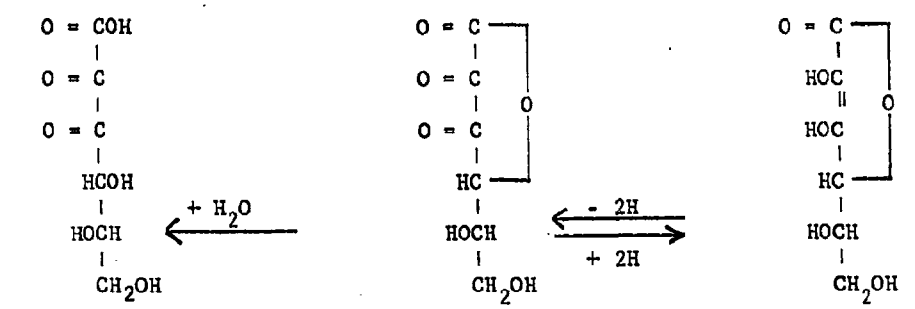
D-Glucose

D-Glucurono-γ-lactone

L-Gulono-γ-lactone

Enzyme not present in man, other primates, guinea pig, fruit-eating bat and Indian bulbul)

L-Gulono γ-lactone oxidase (liver)



L-Diketo-gulonic acid

Inactive

Dehydro-ascorbic acid

L-ascorbic acid

Antiscorbutic activity

In the absence of stresses, dental and skeletal structures are generally normal in animals on intakes of 2 to 3 mg. daily. However, if stress is applied, as by injections of diphtheria toxin, 3 mg. are insufficient for optimal development and advantages of intakes up to 8 to 10 mg. daily can be demonstrated. Sublethal injections of diphtheria toxin, furthermore, result in secondary necrotic effects at the sites of injection and cause large losses of ascorbic acid from tissue reserves in animals fed 0.5 to 3 mg. of the vitamin[8]. These results occur but are less evident on intakes of 5 to 10 mg. daily. In addition, intakes of 8 to 10 mg. daily over successive generations improve the number and vitality of the young and the survival of the parent. Larger intakes that no longer show beneficial effects in tissues appear to improve the efficiency of utilization of other nutrients. The microflora of the intestinal tract affect the utilization of ascorbic acid inasmuch as guinea pigs in a germ-free environment are more resistant to scurvy than conventional animals.

Fewer data are available in the case of infants and human adults. The intake of an infant nursing at the breast of a well-fed mother may range from 20 to 40 mg. daily. Infantile scurvy is not observed on intakes above 10 mg. Lower values can occur on diets of unsupplemented evaporated cow's milk or of improperly pasteurized whole milk. Embryonic synthesis of ascorbic acid does not occur.

Crandon studied the onset of scorbutic symptoms in himself while subsisting for a 6-month period on a diet devoid of ascorbic acid[9]. Plasma levels decreased to zero after 41 days and buffy coat (leucocytes and platelets) levels to zero after 121 days. Hyperkeratotic papules appeared over the buttocks and calves after 132 days and perifollicular hemorrhages after 161 days. Good healing occurred in an experimental incision in the midback made after 85 days, but in a similar wound made on the 182d day there was no evidence of healing or of formation of intercellular substance. Anemia did not occur despite blood loss by venesection totaling 6 liters. The gums became slightly "boggy" but the gingivae remained normal. All signs disappeared rapidly after administration of the vitamin.

Adults are protected from the onset of scurvy by slightly less than 10 mg. daily and, if deficient, some therapeutic effect of this amount can be observed[10].

C. Distribution in Tissues.

Ascorbic acid is widely distributed throughout the tissues of the body, both in animals in which synthesis occurs and in animals of the susceptible group provided adequate amounts are consumed in the diet. The direct relation between the dietary intake and tissue levels in the guinea pig is illustrated in Table 1[7]. The largest concentration is in the adrenal gland and high levels are found in the liver, spleen, and brain. It is to be noted that the observed tissue levels do not necessarily represent "saturation" levels. The level in the blood has been found to serve as an accurate laboratory index of the dietary intake, except for intakes considerably in excess of normal requirements. The blood ascorbic acid consists of 2 fractions, one in the plasma and one in the "buffy coat," the leucocytes and the platelets (Table 2). The latter fraction appears to parallel the quantity found in the liver.

It is assumed that the tissue distribution in man is similar to that found in the guinea pig. An average renal threshold value in human plasma is 1.4 mg.%. Maximum buffy coat values are 28 to 32 mg.%. This level is believed to represent a state of tissue saturation with the vitamin.

TABLE 1

Relation of intake (mg. per day per 100 gm. body weight) to tissue ascorbic acid concentration (mg.%) in guinea pigs on diets for 26 or more days. (5 or more animals per group)

Intake	Whole Blood	Liver	Spleen	Kidney	Adrenal	Muscle	Brain	Heart
0.18	0.09	0.79	2.3	0.6	4.5	0.2	3.1	0.3
0.53	0.14	2.46	8.0	1.7	16.8	0.5	5.6	1.1
0.70	0.14	3.75	12.8	2.5	28.5	0.8	8.4	2.2
0.90	0.19	4.93	17.1	3.1	37.7	0.9	10.5	2.6
1.23	0.26	7.68	20.9	4.4	53.2	1.1	13.2	3.4
1.53	0.28	9.71	25.2	5.1	59.3	1.3	14.6	3.8
1.83	0.36	11.59	26.0	5.6	73.9	1.6	15.2	4.6
3.44	0.54	15.45	33.1	7.9	110.5	2.2	18.4	5.5
Cabbage	0.75	23.77	42.8	9.1	118.6	2.4	18.6	7.5

From C. A. Kuether, I. R. Telford and J. H. Roe, J. Nutrition 28, 347-358 (1944)[7].

TABLE 2

Relation of ascorbic acid level in blood plasma and "buffy coat" to intake in normal men. Experimental period, 8 months; 25 men per group

Daily intake mg.	Plasma mg. per 100 ml. range*	Plasma mg. per 100 ml. average	Buffy coat mg. per 100 gm. range	Buffy coat mg. per 100 gm. average
8	0.05 - 0.4**	0.18 ± 0.01***	4-19	11.9 ± 0.4***
23	0.1 - 0.4	0.20 ± 0.01	7-19	12.9 ± 0.4
78	0.2 - 1.7	0.79 ± 0.04	20-33	24.2 ± 0.6

*Estimated from authors' graph.
**Two subjects having less than 0.1 mg.%.
***Probable error of mean.

Modified from O. H. Lowry, O. A. Bessey, M. J. Brock, and J. A. Lopez, J. Biol. Chem., 166, 111 (1946)[11].

D. Tissue Reserves in Man.

Continued ingestion of 100 to 200 mg., or more, of ascorbic acid daily by most normal subjects results in average plasma levels of 1.4 mg.%, white cell levels of 28 to 32 mg.%, and saturation of the tissues. One-half or more of these body stores may be lost over a period of weeks in otherwise normal individuals without any evidence of physiological impairment. From this viewpoint, ascorbic acid is stored during periods of high intake.

The response of plasma and leucocytes to dietary intake is illustrated by the data on normal men maintained for 8 months on 8, 23, or 78 mg. of ascorbic acid daily (Table 2)[11]. Noteworthy is the large individual variation found in the group with a daily intake of 78 mg. It is significant that the range of plasma values extended from a near-deficiency level of 0.2 mg.% to a saturation level of 1.7 mg.%. Obviously, an intake of 78 mg. of ascorbic acid daily was far below the amount required to saturate the tissues in many of the subjects of this experiment. None of the buffy coat values in the 78 mg. group was in the deficiency range, which suggests that these cells are less likely to show extreme, possibly temporary, shifts in concentration than the plasma. No evidences of ascorbic acid deficiency appeared in the group ingesting only 8 mg. daily for the 8-month period.

The data in Table 3 show clearly the gradual depletion of plasma ascorbic acid and, presumably, of tissue reserves if the intake is insufficient to maintain saturation levels[12]. In this instance, the values after 6 weeks on intakes of 70, 53, and 33 mg. daily averaged 73, 45, and 35%, respectively, of the initial plasma levels. The last column in Table 3 also demonstrates the rapidity with which the stores are replenished with sufficiently large intakes of the vitamin. It is assumed that the urinary excretion of 50% or more of the 400 mg. intake is an indication of tissue repletion.

An even more striking example of repletion of depleted tissue reserves of ascorbic acid is shown in Table 4[11]. Subjects receiving only 8 mg. daily for an 8-month period replenished the depleted tissue stores within 4 days when given 2000 mg. daily. Admittedly, this conclusion assumes that ascorbic acid moves rapidly into tissues from the plasma.

These and other observations are consistent with the conclusions that plasma and tissue levels of ascorbic acid are subject to wide variations in normal individuals, that these variations are related to intake, and that marked depletion of body stores may occur without evidence of associated interference with normal body functions. The findings throw no light on the question of a possible diminished resistance to stress in subjects whose tissues are appreciably less than saturated with the vitamin.

E. Safety of Ascorbic Acid.

No findings have been reported that suggest that ascorbic acid has toxic effects when administered in amounts greatly in excess of quantities required for saturation of the tissues. This is well illustrated by the data in Table 5 which show the serum and buffy coat levels and the urinary excretion of ascorbic acid in 4 subjects whose intake was 1000 mg. per day for 98 days[13]. Saturation of the tissues for this extended period was not associated with any deleterious results.

Because oxalic acid is one of the normal end products of the catabolism of ascorbic acid, the question of possible aggravation of oxalate stone formation in individuals

TABLE 3

Relation of ascorbic acid level in blood plasma and of urinary excretion to intake in normal men and women.
Experimental period, 6 weeks; preliminary "saturation" by ingestion of 400 mg. daily for 4 days.

Daily intake	Average plasma level		Days required for saturation* in 7th week on daily intake of 400 mg.
	1st week	6th week	
mg.	mg. per 100 ml.		
70	1.07	0.86	2
	0.76	0.67	2
	0.89	0.50	3
	1.06	0.63	2
	0.83	0.66	2
53	1.22	0.63	3
	1.03	0.42	5
	1.04	0.46	4
	1.25	0.53	3
33	1.00	0.34	4
	0.96	0.36	4
	0.93	0.28	5
	0.93	0.31	4

*50% or more of daily intake excreted in urine.

From J. E. Haines, A. M. Klosterman, H. M. Hauck, M. A. Delaney, and A. B. Kline, J. Nutrition 33, 479-489 (1947)(12).

TABLE 4

Restoration of tissue deficit of ascorbic acid in 4 subjects given 2000 mg. daily after an 8-month period on an 8 mg. intake daily.

Days of 2000 mg. intake	Urinary excretion mg. per day	Retention mg. per day
1	11	1528
2	980	659
3	1430	136
4	1539	none

Total retained - 2323

From O. H. Lowry, O. A. Bessey, M. J. Brock, and J. A. Lopez, J. Biol. Chem. 166, 111 (1946)(11).

TABLE 5

Blood and urine levels of ascorbic acid in 4 subjects receiving 1000 mg. daily for 98 days.

Day	Plasma mg.%	Buffy coat mg.%	Urine mg.
0	1.22	27	-
5	1.81	28	817
21	1.79	30	804
39	1.82	28	714
98	1.64	28	822

From O. H. Lowry, O. A. Bessey, and H. B. Burch, Proc. Soc. Exptl. Biol. Med. 80, 361 (1952)(13)

receiving excess amounts of the vitamin is pertinent. This is not believed to be a hazard because oxalate does not increase in the urine significantly as the intake of ascorbic acid increases. This end product of ascorbate metabolism in man appears to reflect the actual turnover of the vitamin and, as will be noted later, is probably under 20 mg. per day for normal men. Whether or not oxalate formation from ascorbate increases during stress is not known.

F. The Rate of Utilization of Ascorbic Acid in Man.

The determination of the rate of urinary excretion of carbon-14-labeled ascorbic acid and of its labeled oxidation products, especially of oxalic acid, after the oral administration of 20μc of L-ascorbic-1-C^{14} acid (specific activity of 1.35 MC per mM) permits the estimation of the actual utilization of the vitamin. In such experiments on 6 healthy adult men it was noted that utilization was correlated with lean body mass rather than with total body weight and that utilization occurred at the rate of 0.207 mg. per day per kilogram of fat-free body weight[14]. On this basis an 80 kg. man with a lean body mass of 70 kg. would utilize 14.5 mg. of the vitamin daily. From these same experiments, the total body ascorbate was estimated as 32 to 34 mg. per kilogram of fat-free body weight. For the 6 men used in this study, the total body ascorbate ranged from 1.3 to 2.4 gm.

The data in Table 6 illustrate the findings in a similar study of turnover rates in 3 hospital subjects given ascorbic-1-C^{14} acid[15]. The estimates of the size of the body pool of ascorbate in these 2 investigations are 33 mg. per kilogram of body weight (fat-free) and 22 mg. per kilogram of body weight (total), respectively, values that are somewhat more than one-half of the 50 mg. per kilogram calculated from intakes required to saturate the tissues[11].

G. Ascorbic Acid and the Formation of Collagen.

An impaired formation of collagen in ascorbic acid deficiency is the basis of the effects commonly observed in the gums, teeth, joints, bones, and vascular system. In fact, the failure of production and maintenance of normal intercellular substance in scurvy involves the collagen of all fibrous tissues, including that required in the healing of wounds. The protein collagen is produced by fibroblasts including osteoblasts and chondroblasts. It is synthesized intracellularly as tropocollagen and appears first as unstriated filaments. The characteristic collagen fibers are formed during or immediately after extrusion of the fibrils from the cell. In addition to the development of the unique fibrous form of the protein, the production of collagen requires the synthesis of hydroxyproline as well as the 2 synthetic reactions that are common to the biogenesis of all protein, namely, amino acid activation and peptide bond formation. The high level of hydroxyproline, approximately 14%, serves to distinguish collagen from other proteins and, in fact, is used as a measure of the collagen content of tissues. The claim that a nonfibrous, hydroxyproline-poor protein precursor of collagen accumulates during scurvy has not been substantiated. Ascorbic acid acts at the cellular level to increase collagen synthesis and this role appears to involve the hydroxylation of proline and, presumably, of lysine.

The role of ascorbic acid in collagen formation has been demonstrated by studies of growth and of wound healing in normal and scorbutic guinea pigs[16, 17]. These investigations have been greatly aided by the observation that the subcutaneous implantation of polyvinyl sponges or of small portions of carrageenan (Irish moss) in

TABLE 6

Body pool and turnover rates of L-ascorbic acid in man, guinea pig, and rat.

	Plasma level mg.%	Half life days	Pool mg./kg.	Turnover time days	Turnover rate mg./kg./day
Subject No. 1*	0.35	13	26	19	1.4
Subject No. 2*	0.54	15	21	22	1.0
Subject No. 3*	1.00	20	19	29	0.66
Guinea pig	-	4.0	54	5.8	9.3
Rat	-	2.9	107	4.1	26

*No. 1, multiple lipomatosis; No. 2, multiple sclerosis; No. 3, recurrent breast cancer with metastases.

From L. Hellman and J. J. Burns, J. Biol. Chem. 230, 923 (1958)[15].

normal guinea pigs results in rapid infiltration with fibroblasts and in the production of 12 to 20 gm. of granulomatous tissue in the sponge during a 14-day period. The granuloma may contain 12 to 16% of collagen if the diet is adequate but only 2% if the diet is scorbutic. The collagen deficit results if the animal is deprived of the vitamin at the time of the injection of the carrageenan and, hence, occurs before the usual inanition and deficiency signs appear. Of special interest is the fact that a brei prepared from a scorbutic granuloma shows an in vitro formation of collagen when supplemented with ascorbic acid.

Whether or not ascorbic acid is essential for the maintenance of preformed collagen has been a controversial question. Some collagen is formed in wounds under the most drastic conditions of ascorbic acid deficiency but it is still uncertain if tissue depletion in these instances is complete. The evidence suggests that slow collagen biogenesis may occur during growth in the absence of dietary ascorbic acid. Collagen appears in cultures of chick embryo fibroblasts and may be demonstrated in the skin and carcass of young guinea pigs on diets devoid of the vitamin. No wound healing occurs in these animals and it must be concluded that the accelerated synthesis of collagen required for healing and also characteristic of the sponge implantation experiments is dependent on an adequate supply of tissue ascorbic acid. Developmental collagen, synthesized under normal conditions, is inert and remains resistant to breakdown in tissues after the onset of scurvy. On the other hand, the deprivation of ascorbic acid in guinea pigs with active sponge granulomas or with recently healed wounds results in the partial disappearance of reparative collagen.

The fundamental defect in collagen formation in ascorbic acid deficiency is the inability to convert proline into hydroxyproline, without which the building of the collagen molecule is impossible. The evidence favors the hypothesis that ascorbic acid is the precursor of monodehydroascorbic acid which, as a free radical, participates actively in hydroxylations. D-ascorbic acid, the unnatural optical isomer of the vitamin, also exhibits this property. As will be noted in following sections, the duplication of some of the effects of ascorbic acid by certain compounds exhibiting a pronounced activity in oxidation-reduction systems in common with ascorbate has clouded the identification of its specific role in metabolism. This circumstance, however, does not deny the fact of the indispensable nature of the ascorbate function, whatever it is, or the fact of the dependence of man on a dietary source of this naturally occurring nutrient.

H. Ascorbic Acid, the Hydroxylation Reaction in Collagen Biosynthesis, and a Possible Role in an Electron Transport System.

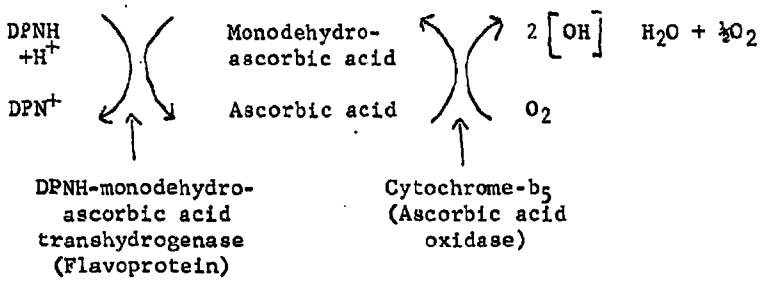
Collagen is the primary constituent of white fibrous connective tissue and occurs, therefore, in considerable amounts in all tissues of the body. It is a stable protein with a very low rate of turnover, yet it is readily converted in vitro into gelatin. It is an unusual protein, also, because 3 amino acids, glycine, proline, and hydroxyproline, make up over one-half of the molecule. Tryptophan and cystine are absent and the levels of methionine, phenylalanine, and tyrosine are low. Collagen is estimated by measuring the concentration of hydroxyproline (14.1%). The latter is principally 4-hydroxyproline but a small amount of the 3-hydroxy compound is present, as is also hydroxylysine.

Normal biosynthesis of collagen requires hydroxyproline and hydroxylysine, neither of which is normally supplied from exogenous sources. The endogenous supply of these hydroxylated amino acids fails in the absence of ascorbic acid. It is not clear

whether this failure indicates that the vitamin plays a direct and specific role in the hydroxylation reaction or whether it has some other unrecognized function in collagen formation. The former appears a reasonable conclusion in view of the evidence that associates ascorbate with hydroxyproline formation as well as with other hydroxylation reactions to be discussed later.

The mechanism of conversion of proline to hydroxyproline is not clear but it appears that the hydroxylation reaction occurs in the preliminary steps of peptide biogenesis. Noteworthy is the fact that the administration of labeled hydroxyproline does not result in the appearance of the marked amino acid in the collagen molecule whereas labeled hydroxyproline is found in collagen after administration of labeled proline. Proline, then, is the precursor of hydroxyproline and these findings suggest not only that hydroxylation is dependent on ascorbate but also that this reaction does not precede the initial steps in the building of the hydroxyproline segments of the peptide chains of collagen[16a].

Staudinger has reported evidence for an ascorbic acid-dependent enzyme in microsomes from pig and beef adrenals which catalyzes the oxidation of reduced diphosphopyridine nucleotide (DPNH). The enzyme appears to be a flavoprotein with active sulfhydryl groups and is insensitive to cyanide and to actomycin A[18]. The product of the oxidation of ascorbic acid is not dehydroascorbic acid but the intermediate, monodehydroascorbic acid. Staudinger proposed that ascorbic acid is a component of the electron transport system illustrated as follows:



However, the evidence does not support the conclusion that ascorbic acid plays a major role in electron transport in animal tissues. Staudinger's system has not been definitely established and, in fact, the properties of cytochrome-b_5 suggest that it might well function in the chain before, rather than after, ascorbic acid. Furthermore, such a series of reactions in microsomes cannot be quantitatively important because these particles account for less than 10% of the electron transport believed to occur in mitochondria. Its significance in mitochondria is entirely unknown, but it is known that less than 10% of the tissue ascorbic acid is in the mitochondria. Although the total ascorbate concentration in most tissues under normal conditions is sufficiently high to suggest the possibility of the operation of an "active" transport system involving ascorbate, there are no data on the in vivo tissue levels of the apparently limiting active molecule, monodehydroascorbic acid. Interestingly, D-ascorbic acid also functions in these experimental systems.

Despite the lack of support for a major role for ascorbic acid in energy metabolism, the evidence does justify the tentative conclusion that a reaction of the type proposed may be a specialized oxidation-reduction mechanism for the microsomal formation of certain hydroxylated products.

I. Ascorbic Acid and Other Hydroxylation Reactions.

1. Hydroxylation and tyrosine metabolism.

Premature infants, normal infants receiving less than completely protective amounts of ascorbic acid, and partially scorbutic guinea pigs excrete para-hydroxyphenylpyruvate (p-HPP) in the urine following the administration of the amino acids phenylalanine or tyrosine. p-HPP is a normal intermediate in the metabolism of tyrosine, but its accumulation in the blood in such quantities as to result in the appearance of significant amounts in the urine is associated only with an insufficient supply of ascorbic acid. The excretion of urinary p-HPP also occurs in rats fed excessive levels of tyrosine and, in this case, it would appear that the animal's ability to synthesize ascorbic acid had been exceeded.

The normal sequence of reactions in metabolism includes the conversion of tyrosine to p-HPP by a transaminase and of p-HPP to homogentisic acid by a hydroxylase. The latter step includes the further hydroxylation of the para-hydroxyphenyl ring. p-HPP accumulates in ascorbic acid insufficiency because the first of the above reactions proceeds more rapidly than the second[19]. This result is not due to a decrease in the amounts of the hydroxylase but to an inhibition of its activity. The critical part of this mechanism appears to be a physiological action of ascorbic acid, in greater than antiscorbutic concentrations, in maintaining the essential iron component of a specific hydroxylase, para-hydroxyphenylpyruvate hydroxylase, in the active reduced or ferrous form. Without adequate ascorbic acid, the iron of the hydroxylase in animals given too much tyrosine is oxidized to the ferric state and the accumulation of p-HPP results from the inactivation of the enzyme. The inactivated enzyme can be reactivated *in vitro* and *in vivo* by ascorbic acid. The enzyme normally exists in the ferric form in very young rats and it can be activated, in part, by reduction by ascorbic acid *in vitro* and, possibly, *in vivo* also.

These findings support the conclusion that ascorbic acid has a physiological role in maintaining the reduction of certain strategic forms of iron, a role that varies with age and diet. This function has not been shown to depend on ascorbic acid as an essential part of a specific coenzyme. Its action in laboratory studies appears to be nonspecific because the accumulation of p-HPP can be prevented also by D-glucoascorbic acid, by D-isoascorbic acid, and by other reducing agents such as hydroquinone, para-aminophenol, para-phenylenediamine, and 2,6-dichlorophenolindophenol.

Pteroylglutamic acid (folic acid) and its triglutamic acid homologue also abolish the urinary excretion of p-HPP in tyrosine-fed scorbutic guinea pigs[20]. Folic acid has no antiscorbutic activity and its mode of action in facilitating the catabolism of p-HPP is unknown. It may be that the folic acid-folinic acid system also aids in maintaining the iron of the hydroxylase enzyme in the ferrous state.

2. Hydroxylation and tryptophan metabolism.

The formation of the vasoconstrictor, 5-hydroxytryptamine or serotonin, from the amino acid tryptophan is a two-step reaction involving the hydroxylation of tryptophan at position 5 and decarboxylation of the resulting 5-hydroxytryptophan. Extracts of kidney and of intestinal mucosa of normal rats and guinea pigs contain a tryptophan-5-hydroxylase which calatyzes the formation of 5-hydroxytryptophan[21]. The reaction proceeds either under aerobic or anaerobic conditions and requires the presence of cupric copper and of L-ascorbic acid or a similar reducing compound. D-ascorbic and isoascorbic acids are active but 2,6-dichlorophenolindophenol is not. Thus the specificity of the role of ascorbic acid is uncertain.

3. Other hydroxylations.

Participation of ascorbic acid in the formation of hydroxylated steroid hormones has been suggested[18]. However, no such reaction has been demonstrated for hydroxylation at carbon-21 in the case of progesterone and its derivatives, although a requirement for reduced triphosphopyridine nucleotide and oxygen was observed in microsomal preparations of beef adrenal[22]. Furthermore, no suggestion of a role of ascorbic acid in the enzymatic hydroxylation of steroids was emphasized in a recent review of this subject[23].

On the other hand, a nonenzymatic hydroxylation of aromatic compounds by ascorbic acid, ferrous iron, and oxygen has been studied[24a, 24b]. The hydroxyl group generated by this system is electrophilic and hydroxylation occurs at electronegative sites on aromatic or heterocyclic rings. Of special interest is the observation that the same hydroxylated products are formed in vivo if the substrates are administered and that the rate of hydroxylation in vivo is markedly reduced in guinea pigs depleted of ascorbic acid.

J. Ascorbic Acid and Wound Healing.

Patients with hemorrhagic shock, traumatic injuries and infection[25b], and patients with burns[25c] are reported to benefit from daily supplements of 1-2 gm. of ascorbic acid, 10-20 mg. each of thiamine and riboflavin, and 150-250 mg. of nicotinic acid in a diet enriched with protein, calories, yeast, crude liver extract, and vitamins A and D. Because the relatively large supplements of ascorbic acid reversed or prevented decreases in plasma ascorbic acid, apparent decreases in tissue saturation, and increases in urinary excretion of the vitamin, Levenson et al. considered that these patients exhibited "biochemical" scurvy. Specific tests of "physiological" scurvy were not made although certain of the patients were quite scorbutic-like in at least two regards, viz., the excessively high intake of ascorbic acid required to raise the plasma level above deficiency levels and the apparent improvement in the survival of skin grafts associated with increased plasma ascorbate.

Subsequently, Levenson et al.[25d] studied the healing process in scorbutic and normal guinea pigs. It was first determined that 2 mg. daily was the minimal amount of ascorbic acid that would support growth and normal healing of experimental incisional wounds in these animals. In the absence of the daily supplement of 2 mg. during the postoperative period, the healing process was grossly impaired. In animals with abdominal incisions and back burns, the healing was markedly abnormal and was histologically indistinguishable from that observed in scorbutic animals even though the burned animals continued to receive 2 mg. of ascorbic acid daily. The failure of the ascorbate supplement to protect the burned guinea pigs was shown to be unrelated to a decreased food intake or to the presence of as much as 10 mg. of cortisone administered subcutaneously daily during the postoperative period. Healing was normal when the ascorbic acid supplement was increased to 100 mg. daily.

Studies on the metabolism of tyrosine by Levenson's group have yielded further evidence that guinea pigs subjected to experimental burns behave biochemically and physiologically like scorbutic animals even though given supplements of ascorbic acid previously demonstrated to be sufficient to support normal healing of incisional wounds[25e]. Loading with tyrosine (0.75 mg. per gm. of body weight) did not result in an increased urinary excretion of para-hydroxyphenylpyruvic acid (p-HPP) in animals receiving the daily 2 mg. supplement of the vitamin. In burned animals with the same intake of ascorbic acid there was a marked excretion of p-HPP. Excretion of p-HPP

was prevented in animals receiving 100 mg. of the vitamin daily. The minimum protective intake was not determined. The evidence appears convincing, however, that the requirement for ascorbic acid is increased in guinea pigs subjected to severe burns.

Considerable attention has been paid to the abnormal healing process in scorbutic animals in an attempt to identify the specific failure. For this purpose, guinea pigs with both experimental incisions and implanted polyvinyl sponges have been prepared and maintained on adequate or scorbutic diets for a 14-day period. Evaluation of the relation of ascorbate to the healing process was based on a histopathological examination of the tissues, a measurement of the tensile strength of the scar, and a determination of the collagen formation in the sponge based on the estimation of hydroxyproline. In scorbutic animals, the incisional scar consisted of a bridge of surface epidermis covering bloody granulation tissue. The sponges, in turn, appeared to float in a pool of unclotted blood. In neither case was there any indication of an inhibition of fibroblasts which were present in apparently normal numbers. However, there was failure of normal fiber formation and the decreased tensile strength of the incisional scar was proportional to the decreased hydroxyproline concentration of the sponge granuloma. These studies clearly demonstrated the essential requirement of the vitamin for normal healing mechanisms but have not explained the exact role of ascorbic acid in the production of collagen by fibroblasts.

Granulomas in scorbutic guinea pigs are characterized not only by the absence of collagen but also by the presence of a large excess of mucopolysaccharides, principally hyaluronic acid[16a, 16b]. Both changes are reversed by ascorbic acid. Increased hyaluronic acid is also a characteristic of impaired wound healing in scorbutic animals. Reports are conflicting on the question of an effect of ascorbic acid on the incorporation of sulfate into sulfated mucopolysaccharides. The excessive accumulation of mucopolysaccharides in scorbutic tissue might well interfere with the orderly arrangement of collagen fibers in the "ground substance" and, thus, explain the degradative histologic changes observed in collagen during scurvy. It is reasonable to assume that these profound changes in the chemical nature of the connective tissue formed in a wound in a partially deficient animal are related to the lack of resistance of "healed" wounds to rupturing or tearing apart. It has long been recognized that wounds may open during a subsequent exposure to severe ascorbic acid deficiency.

During the process of healing of experimental incisions in guinea pigs, ascorbic acid accumulates in the newly formed connective tissue[26]. The concentration is highest at the central core of the scar and equals the level characteristic of tendons, viz., 6 mg.%. The level found 1 to 2 mm. from the wound is somewhat less (4.7 mg.%) and the content of muscle tissue decreases with distance from the scar, being 1.7 mg.% at 3.0 cm. In these animals, the amounts in distant muscles and in blood were 1.3 and 0.3 mg.%, respectively. Even with a relatively small wound, the shift in distribution of tissue ascorbate results in a slower rate of expiratory removal of $C^{14}O_2$ after administration of ascorbic-1-C^{14}[27].

The findings of the wound healing experiments suggest strongly that the simultaneous occurrence of experimental incisions and of severe burns in animals results in unusual demands on the tissue reserves of ascorbic acid and that healing is impaired in the absence of these reserves. Whether the increased need is due primarily to an accelerated catabolism of ascorbate, to the accumulation of the vitamin in the damaged or healing tissue, or to loss in the exudate from the surface of the burned tissue has not been determined with certainty. The concentration of ascorbic acid in early edema fluid of burns has been shown to be similar to the concentration in blood plasma[28].

K. Ascorbic Acid and the Vascular System.

Microscopic examination of the mesentery of scorbutic guinea pigs under carefully controlled laboratory conditions reveals a marked sluggishness of blood flow in vessels that are greatly dilated, that show petechial hemorrhages, and that demonstrate an extraordinary loss of sensitivity to amounts of epinephrine which are stimulatory in normal animals[29]. The dilation, the hemorrhage, and the insensitivity to epinephrine are observed only in the venular portion of the capillary bed where the smooth muscle cells of the vessel wall are discontinuous and where there are extensive areas having only endothelium, ground substance, and collagen fibrils in the venular wall. It is pertinent that these areas are not normally under the control of the nervous system but are under humoral control, that is, control by hormones, electrolytes, and other constituents of the blood. Changes in bleeding time or in coagulability of blood do not occur and the administration of flavonoids, such as rutins and hesperidins, to the scorbutic animals does not alter the vascular responses.

In addition, the scorbutic state in guinea pigs is characterized by a marked reduction in the resistance to severe blood loss. Hemorrhage that is withstood readily by normal animals results in fatal shock in the deficient animals. Normal and scorbutic guinea pigs, like other animals, show the appearance of the vasodepressor ferritin in the blood stream with the onset of irreversible shock. However, scorbutic animals lack the unidentified protein material, the so-called vasoexcitor substance, that appears in the blood of normal guinea pigs subjected to severe blood loss. Whether the vasoexcitor is a cause or effect of resistance to shock is not known, but it is suggestive that this beneficial factor is not found in ascorbic acid deficiency and that its absence coincides with the more rapid appearance of ferritin in the bloodstream.

These results indicate clearly that a primary physiological defect in the scorbutic guinea pig is the reduced ability of the vascular system to maintain blood pressure by the normal constriction of blood vessels. The data suggest strongly that ascorbic acid has a dynamic role in maintaining peripheral vascular tone and reactivity and that the failure of this role is the cause of the capillary hemorrhages that characterize the scorbutic condition. It is difficult to relate this remarkable action of ascorbic acid to the maintenance of the integrity of collagen and of connective tissue. Obviously, the exact mechanism of the deterioration of the venular wall remains to be clarified.

L. Ascorbic Acid and Transport of Iron.

The favorable influence of ascorbic acid on the absorption of dietary iron from the intestinal tract is ascribed to the reducing action of the ascorbate and, accordingly, to its reduction of ferric iron to the absorbable form, ferrous iron. A unique and more specific effect of ascorbic acid on the transfer of serum iron into liver tissue has been reported[30].

Transferrin is the protein-iron complex in plasma and ferritin is the protein-iron complex in liver. In both materials iron is in the ferric form. Transferrin iron becomes liver ferritin iron in in vitro experiments at pH 7.4 in the presence of both ascorbic acid and adenosine triphosphate (ATP). Neither ATP nor ascorbic acid is effective alone. Oxygen is required and the transfer is inhibited by inhibitors of respiratory enzymes and of oxidative phosphorylation. It is presumed that ascorbic acid reduces transferrin iron at the cell wall, but only in the presence of ATP, and that iron is released, probably in the form of an iron-ATP complex which

then enters the liver cell and becomes ferritin ferric iron. Presumably, sulfhydryl groups of ferritin participate in the reactions.

Ascorbic acid cannot be replaced by the usual biochemical reducing agents but can be replaced by glucoascorbic acid and by dihydroxymaleic acid. Significantly, the incorporation of serum iron into ferritin of the liver and of the spleen is markedly reduced in scorbutic guinea pigs.

M. Ascorbic Acid and Infections.

There is general agreement that in most instances nutrition and infection interact synergestically[31]. Ascorbic acid concentrations in tissues and body fluids are generally reduced by infections in both human subjects and experimental animals. Scurvy, in cases of borderline intake, may be precipitated by an infection. For example, Hess in 1917 concluded that the appearance of scurvy in infants in low-income families in New York City was precipitated by an infectious disease such as "grippe"[32]. Tubercular infection hastens the onset of scurvy in guinea pigs[33]. On the other hand, severe ascorbic acid deficiency increases susceptibility to infection. Reference has been made previously to the increased toxicity of diphtheria toxin in guinea pigs on diets inadequate in ascorbic acid[8]. Scorbutic animals show decreased phagocytic activity[34]. An exception to the general rule is the antagonism to P. knowlesi infection observed in scorbutic monkeys[35].

Of particular interest are the comparisons of resistance or susceptibility to ascorbic acid deficiency in conventional and germ-free guinea pigs[25a]. On scorbutic diets, germ-free animals survived an average of 44 days compared with 27-day survival for the conventional animals. Furthermore, the deficiency symptoms were more severe in the latter group. A possible explanation is the increased rate of loss of the initial tissue supplies in the conventional animals because of destruction of the vitamin in intestinal secretions by microorganisms.

Despite the many studies on scurvy and infection which have shown conclusively the advantage of adequate intakes of the vitamin, there is an almost complete lack of support for the view that additional benefit is provided by supplements of ascorbic acid greater than amounts commonly accepted as adequate.

N. Ascorbic Acid and Tolerance to Cold.

1. Animals

Exposure of warm-blooded animals to cold temperatures normally results in increased heat production associated with shivering, then followed and replaced, in part, by a chemically regulated production of heat, nonshivering thermogenesis. Hormones of the thyroid and adrenal glands are involved directly in the increased production of heat and the pituitary may be involved indirectly through the thyroid-stimulating hormone (TSH) and the adrenocorticotropic hormone (ACTH).

The orderly production of increased heat sufficient to maintain body temperature in a cold environment is a protective mechanism known as acclimatization. Animals that are unable to respond to continued cold in this way cannot survive exposure to low temperatures that are well tolerated by acclimatized animals.

Ascorbic acid has been shown to play a significant role in the development of tolerance to cold in rats, guinea pigs, and monkeys. Its mechanism of action is not known, but it appears to function peripherally in tissues rather than on the thyroid or pituitary glands. It may act on the adrenal directly, however, and it is

of interest that the stress phenomenon consists of an action of ACTH that results in the immediate movement of ascorbic acid out of the adrenal cortex into the bloodstream accompanied by the elaboration of both medullary catecholamines and cortical steroids. Whether or not the adrenal ascorbic acid participates in the biogenesis of adrenal hormones is not known, but its involvement with the metabolism of phenylalanine and tyrosine, especially with the hydroxylation reaction, makes this suggestion an attractive possibility. Nevertheless, the primary function of ascorbate in acclimatization may be limited to a conditioning action in tissues so that the calorigenic effect of thyroxin and of the epinephrines is enhanced.

These general conclusions are based on numerous investigations[36]. The administration of relatively large amounts of ascorbic acid has been shown to be beneficial in rats, guinea pigs, and monkeys in cold environments. Guinea pigs failed to survive exposure to cold unless supplied ascorbic acid considerably in excess of intakes known to be adequate at normal temperatures[37]. Frostbite and lowered body temperature in rhesus monkeys were less marked during exposure to severe cold (-20° C.) if the animals were given an excess of ascorbic acid and were preconditioned, in part at least, by previous maintenance at 10° C. rather than at room temperature[38]. Tissue levels of ascorbic acid increased in rats and guinea pigs exposed and acclimatized to cold[39]. The increases were less if ascorbic acid was administered and, without supplementary ascorbate, tissue levels decreased in animals in which acclimatization did not occur. Whether animals synthesized the vitamin or not, all that developed tolerance to cold reacted in the same manner, viz., with retention of ascorbic acid in the tissues[40].

It has long been recognized that acute exposure to cold stimulated the pituitary-adrenal system with a resulting increased secretion of the adrenocorticotrophic hormone (ACTH), a hypertrophic enlargement of the adrenals, a release of cortical and medullary hormones, a decrease in cortical cholesterol, and a decrease in cortical ascorbic acid[41, 42]. The explanation of this change in adrenal ascorbate and its relation to the production, secretion, and effect of adrenal hormones have been intensively investigated[43, 44]. The adrenal ascorbic acid has been accounted for in the blood and the transfer was found to precede the appearance of increased corticosterone[45]. The shift in adrenal ascorbate induced by ACTH may serve as a "trigger" for the release of cortical hormones or the vitamin may be involved in the biosynthesis of the hormones.

The typical enlargement of the adrenals by cold stress was completely prevented in rats and guinea pigs by the administration of ascorbic acid[46]. Katsh et al. concluded that adrenal hypertrophy and increased adrenal weight were indices of the heightened adrenal activity caused by hypothermia[47]. A synergistic influence of ascorbic acid on ACTH has been postulated by Dugal who demonstrated such an effect on the secretory activity of the adrenal cortex in hypophysectomized rats given ACTH and ascorbic acid[49b] and by Des Marais who found a similar result <u>in vitro</u> following incubation of adrenal slices[48]. Dugal et al. showed that the administration of ascorbic acid to hypophysectomized rats at room temperature, prior to exposure to cold, so increased the responsiveness of the adrenals that further enhancement by subsequent cold stress and ACTH administration did not occur[49a]. Nicholls has also reported the potentiation of ACTH activity by sodium ascorbate but not by sodium succinate in cold-stressed rats[50].

Biosynthesis of the corticosteroids[51] and of norepinephrine and epinephrine[52] has been demonstrated by perfusion of the isolated calf adrenal. Previous reference has been made to evidence supporting the belief that ascorbic acid favors the hydroxylation of aromatic[24a, 24b] and of hydroxysteroid compounds[18] by the adrenal glands. Of special interest is the observation that norepinephrine is calorigenic in cold-

acclimatized rats but not so in animals maintained at room temperature[53]. Phenylalanine and tyrosine have been shown to be precursors of norepinephrine and of epinephrine[54], and plasma levels of these amino acids were found to be reduced in rats either by dietary additions of ascorbic acid or by exposure of the rats to cold, with the greatest reductions in animals exposed to cold and also given ascorbic acid[55].

Des Marais has studied the relation of ascorbic acid to cortisone and to thyroxine[56]. The catabolic effect of cortisone, which was deleterious to rats in a cold environment, was reversed by the simultaneous administration of thyroxine, an effect that was enhanced by ascorbic acid. The daily administration of 10, but not of 3, micrograms of thyroxine in thyroidectomized rats exposed to cold reversed the catabolic effect of the cortical hormone, increased survival, and maintained the thymus and adrenal weights within normal limits. However, 3 micrograms of thyroxine were effective if administered with ascorbic acid. The vitamin did not influence the effect of a standard dose of thyrotropic hormone (TSH) in hypophysectomized rats maintained at 14° C. or 24° C., indicating that the effect of ascorbate is mediated through the circulating thyroid hormones and not on the thyroid gland. Des Marais has concluded that the action of ascorbic acid is not the same at room and at cold temperatures and that in the cold it, in some unknown way, reduces the requirement for the thyroid hormone.

2. Man

Even though the evidence for an important role in the development of tolerance to cold appears conclusive in both rats and guinea pigs, the question is quite controversial for man. In a prolonged study in which comparisons were made between exposures to -20° F. and 60° F., supplements of vitamins which included 200 mg. of ascorbic acid daily proved of no benefit[57]. The basal diet provided the recommended dietary allowance of the Food and Nutrition Board and the caloric content was sufficient to maintain body weight. The temperature of the living quarters used in the nonexperimental periods was between 72 and 77° F. On the other hand, opposite results were reported in a comparison of supplements of 25 and 525 mg. of ascorbic acid daily in lightly clad men maintained for 13 days in a 24-hour temperature of 59° F.[58]. The basal diet was grossly inadequate in calories and consisted of an emergency-type ration providing only 550 calories per day. No difference was found in rectal temperatures but skin temperatures were definitely higher in the men receiving the larger amount of ascorbate. Subjectively, the group limited to 25 mg. daily complained of tender, swollen feet which made walking difficult. However, in a continuation of this investigation, no differences were found in men on similar regimens but supplied 3400 calories daily.

A test which was designed to determine the influence of ascorbic acid and of other nutrients in military personnel exposed to cold under natural mountainous conditions was inconclusive, partly because the anticipated cold weather did not occur[59a].

During an 11-month period in which a group of 26 men lived in Antarctica, careful examinations were made of plasma ascorbic acid values and gingival health[59b]. One-half of the group worked indoors, one-half out-of-doors. Both groups used the same living quarters and the same messing facilities. Fresh-frozen citrus juices were available, as were supplementary vitamin tablets. The average monthly temperature dropped from +15° F. in January to -35° F. in July and rose again to +5° F. in November. The combined effect of physical activity and exposure to cold resulted in much greater consumption of food by the out-of-doors group, estimated as twice as much,

yet the group gained only one-fourth as much as the indoors group. Throughout the entire period, the plasma ascorbic acid values of the outside group were considerably lower, averaging 0.60 mg.% with a range from 0.54 to 0.68. The indoors group average was 0.91 mg.% with a range from 0.59 to 1.16. The levels for the outside group decreased from an initial value of 0.74 mg.% to 0.47 mg.%, the values continuing to drop in the months in which the temperature was rising. It was not possible to correlate the differences between the groups with any factors other than physical work and cold exposure. However, no difference whatsoever was evident in gingival health.

O. Influence of Drugs on the Metabolism of Ascorbic Acid.

Various drugs having little similarity in most chemical or pharmacological properties have been found to increase the synthesis of ascorbic acid in rats[60, 61]. Associated with this phenomenon is an increased metabolism of both ascorbate and the drug. Among these drugs are representatives of the hypnotics, analgesics, muscle relaxants, antirheumatics, uricosurics, antihistaminics, and carcinogenic hydrocarbons. Interestingly, the pathway of synthesis of ascorbic acid include glucuronic acid as an intermediate. This compound is frequently combined with drugs in animals to form less physiologically active derivatives. However, some drugs, such as borneol, form glucuronides but do not stimulate the synthesis of ascorbate.

Stimulation of ascorbate synthesis has been recognized by the urinary excretion of ascorbic acid and of its end products and by the study of in vitro enzymatic systems. For example, after intraperitoneal injection of 3-methylcholanthrene in rats, the urinary excretion of ascorbic acid increased from a control value of 0.3 mg. daily to 17 mg. and the elevated excretion continued for 50 days. After oral administration of chloretone in rats, the body pool of ascorbic acid was doubled, the turnover rate was increased 8 times, the urinary excretion was increased 25 times, and the metabolism was increased 5 times[62]. Examination of enzymatic systems in the liver of animals pretreated with chloretone showed that the stimulation of the synthetic pathway occurred before, and not after, the formation of glucuronic acid. Particularly affected was uridine diphosphoglucose dehydrogenase, the enzyme catalyzing the oxidation of uridine diphosphoglucose to uridine diphosphoglucuronic acid. The enzyme activity was doubled in the presence of chloretone as a result of an increase in both the activity and the stability of the dehydrogenase[60, 63].

Phenobarbital stimulates the formation of drug-metabolizing as well as the ascorbate-synthesizing systems. After pretreatment with phenobarbital in rats, the duration of physiological activity of the muscle relaxant zoxazolamine was markedly reduced. This effect is believed to result from the increased activity of an enzyme that inactivates zoxazolamine by forming a hydroxylated derivative. Additional evidence for the induction of one or more enzymes by administration of phenobarbital is provided by the blocking effect of ethionine, the antimetabolite of methionine which inhibits protein synthesis, and the overcoming of the ethionine inhibition by methionine. Similarly, ethionine has been shown to prevent the increased synthesis of ascorbic acid resulting from pretreatment with 3-methylcholanthrene[64].

The stimulation of ascorbic acid synthesis by drugs in the rat raises the question of the nature of the effect in animals, such as the guinea pig, in which ascorbic acid synthesis does not occur. One answer has been afforded by the demonstration that the sensitivity of guinea pigs to drugs is increased by the development of scurvy. After 10 to 14 days on a scorbutogenic diet and before external signs of scurvy appeared, the paralysis due to zoxazolamine administration in guinea pigs lasted twice as long as in control animals. At the same time, the rate of metabolic inactivation of the zoxazolamine was significantly reduced[60]. These findings suggest that the enhancing influence of ascorbate on the hydroxylation mechanism is a factor in the more rapid inactivation of zoxazolamine occurring in ascorbate-fed guinea pigs.

An answer of a different type is illustrated by the results of the administration of 3-methylcholanthrene in scorbutic guinea pigs[65]. As has been emphasized previously in this report, the failure of the production of dentin by odontoblasts has proved to be a highly sensitive indicator of ascorbic acid deficiency. The daily administration

of 10 mg. of 3-methylcholanthrene in guinea pigs on a scurvy-producing diet permitted normal dentinogenesis, restored mucopolysaccharides in the gingival tissues, and largely prevented capillary hemorrhages. Nevertheless, this drug was unable to promote survival and it must be concluded, therefore, that it did not support the synthesis of ascorbic acid.

There is no explanation at this time for the fact that certain drugs can enhance reactions that result in increased production of ascorbic acid in animals having this synthetic capacity. Particularly obscure is the understanding of the observation that a drug may mimic some, but not all, of the functions of ascorbic acid in animals that require a dietary source of the vitamin. The conclusion appears inescapable that ascorbic acid has multiple, indispensable, and apparently unrelated roles in metabolism.

P. Ascorbic Acid in Relation to Bones and Teeth.

In the absence of ascorbic acid in man and in those animals unable to synthesize this compound, there are gross abnormalities in connective tissue which are ascribed to the failure of collagen production. There is an associated abnormal formation of the matrix or "ground" substance of mucopolysaccharides and mucoproteins in which the collagen fibers are imbedded. In addition, calcification is faulty and scurvy is characterized, therefore, by improper development of the bones and teeth. Wolbach and Howe confirmed earlier reports dealing with these points and have accurately described the malfunctioning of mesenchymal cells, including odontoblasts, fibroblasts, and osteoblasts, in animals deficient in the antiscorbutic vitamin.

As has been stated previously, the inability of the odontoblasts to manufacture dentin is a constant finding that has proved very useful in experimental studies on scorbutic animals[66]. The ends of the long bones are particularly vulnerable during growth and X-ray analysis of these structures is the most sensitive diagnostic test in infantile scurvy. Typically, there is a band of increased density at the end of the shaft with an area of decreased density nearer the center. Disorganization of calcification is pronounced at the diaphyseal-epiphyseal junction and microscopic fractures associated with bleeding into the subperiosteal space may progress to malformation and separation of the epiphyses. "Beading" of the ribs at the costochondral junctions is noticeable, as is a "gerüstmark" consisting of a region of edematous connective tissue at the ends of the diaphyses.

Attention has been called in the previous section to the fact that the hydrocarbon, 3-methylcholanthrene, supports dentinogensis by odontoblasts in guinea pigs deprived of ascorbic acid. It is of interest to consider this effect in the light of the activity of other compounds reported to show ascorbic acid-like properties in guinea pigs. Usual methods of study involve comparisons of the ability of test substances to equal L-ascorbic acid in promoting growth, survival, and dentinogenesis and in preventing reduction of serum alkaline phosphatase, appearance of extra urinary p-hydroxyphenyl pyruvate (p-HPP), and evidences of abnormalities of calcification in the long bones. Although no substances have been found with physiological activities equal to ascorbic acid and dehydroascorbic acid, many exhibit partial activity. 3-Methyl-L-ascorbic acid has been reported to have less than one twenty-fifth the activity of the vitamin in the phosphatase test[67]. The daily administration of 12 mg. of D-ascorbic acid did not prevent hemorrhages but did permit survival, weight gain, and dentinogenesis[68]. However, the predentin was abnormal in its reaction to mucopolysaccharide stains. 2,6-Dichlorophenolindophenol prevented the substrate-induced inhibition of p-HPP oxidase but was without effect on odontoblasts and on hemorrhage. 3-Methylcholanthrene permitted dentin formation, was partially effective in the prevention of hemorrhage, and ineffective

in preventing disorganized calcification in the long bones.

These results, extraordinary as they are, explain the dilemma faced by investigators in this field. Perhaps, as stated by LaDu and Zannoni[69] in discussing the role of ascorbate in tyrosine metabolism, "Future experiments will reveal instances in which ascorbic acid acts as a conventional vitamin in some of the other biochemical processes that are deranged in scurvy." Disconcerting as it is to find nonspecific duplication of the role of ascorbic acid, especially in dentinogenesis, it is pertinent to note that this compound appears to be a highly important participant in and regulator of numerous metabolic reactions in the animal world generally. From this viewpoint, it is not surprising that there is recurring evidence of numerous roles, some of which depend only on activity in an oxidation-reduction system for which ascorbic acid is admirably fitted. The strange fact is that man and a small minority of other animal species should have a genetic failure of the important enzymatic mechanism which produces this essential, multifunctional component of the tissues and body fluids of most animals.

Q. Role of Ascorbic Acid in Intermediary Metabolism.

In addition to its influence on the formation of hydroxyproline for collagen formation and on other reactions involving amino acids and hydroxylations and to its effect on the absorption and metabolism of iron, ascorbic acid has been implicated in both fat and carbohydrate metabolism. It is involved in the conversion of acetate-C^{14} to cholesterol and other steroids[70] and influences the CoA concentration in the liver[71]. Banerjee et al. reported that the total body cholesterol was increased in scorbutic guinea pigs and that treatment with insulin reduced the cholesterol to a normal level[72]. An examination of plasma lipids in scorbutic guinea pigs showed that supplements of ascorbic acid returned the levels of plasma beta-lipoprotein, beta-lipoprotein cholesterol, nonesterified fatty acids, and plasma triglycerides to normal[73]. Insulin treatment of the scorbutic animals corrected only the fatty acid and triglyceride values. The beneficial effect of lipoic acid on the signs of scurvy in guinea pigs has been ascribed to a protective action of this substance on the vitamin[74].

Rats exposed to cold show a great capacity to resist fatty infiltration of the liver on diets that are hypolipotropic in animals maintained at a normal temperature[75]. Cold-acclimatized rats also retain liver glycogen at high levels during fasting. Masoro has found that cold-acclimatized rats have an increased capacity to oxidize long chain fatty acids and concluded that the absence of hepatic lipogenesis in these animals was not related to an abnormality of carbohydrate metabolism[76]. The respiratory quotient was lowered in rhesus monkeys exposed to cold and this evidence for an increased metabolism of fat was not influenced by ascorbic acid[77]. Thus, the proof of a specific role of the vitamin in fat metabolism is inconclusive and rests largely on the still unclear part that it plays in acclimatization to cold and in supporting the increased activity of thyroxin in animals exposed to low temperatures. With regard to the latter, Beaton et al.[78] have confirmed earlier reports that oxygen consumption is increased in scorbutic guinea pigs and have demonstrated an increased thyroidal uptake of iodine-131 in these animals. The increased thyroid activity in scorbutic guinea pigs was linked to the abnormal metabolism of tyrosine.

Glycogenesis is depressed in scorbutic guinea pigs. Studies of liver enzymes showed that uridine diphosphate glucose pyrophosphorylase, glycogen synthetase, and phosphorylase, the three glycogen cycle enzymes, were unaffected but that the activities of hexokinase and of phosphoglucomutase were diminished. The availability of uridine

triphosphate also appeared to be a limiting factor[79, 80]. The production of insulin has been reported to be decreased in scorbutic guinea pigs and scurvy has also been associated with striking changes in the tricarboxylic acid cycle which are in large part reversed by insulin[81, 82]. The defect in the metabolism of citrate was based on the accumulation of citric and malic acids in the tissues of scorbutic guinea pigs and on the greatly increased excretion of citrate after the feeding of succinic, malic, or citric acids[83, 84, 85]. Studies of components of the Krebs cycle indicated that the failure may not have been in the aconitase system[86] but was more probably between citric acid and alpha-ketoglutaric acid[84]. Current findings point to a diminished activity of oxalosuccinic decarboxylase as well as of succinic, malic, and lactic acid dehydrogenases[84, 87]. These activities were restored in the scorbutic animals by the administration of insulin. Whether or not the observed abnormalities are due primarily to interference with insulin production in scorbutic guinea pigs is uncertain. Clarification of this problem is urgently needed.

It is of interest that a specific antagonist of L-ascorbic acid has not been demonstrated and this adds to the weight of evidence that the vitamin may not function as part of a coenzyme. Glucoascorbic acid was formerly believed to be such an antimetabolite, but it is now believed that it exerts toxic effects that are not identical with scurvy and that are not overcome by L-ascorbic acid[88, 89].

R. Ascorbic Acid and Anemias.

The favorable influence of ascorbic acid on the absorption of iron has been clearly demonstrated[90], as has its participation in iron transport[22] (See Section L). There is also abundant evidence that the vitamin is concerned with hematopoiesis. Anemia has been considered a result of severe scurvy[91], and a number of studies have suggested a relation between ascorbic acid and folic acid in addition to the ascorbic acid-like effect of folic acid in abolishing the urinary excretion of p-HHP in tyrosine-fed scorbutic guinea pigs[20] (See Section I-1). A deficiency of ascorbic acid in monkeys gave rise to a deficiency of folic acid with resulting megaloblastic anemia[92]. Pernicious anemia patients require more liver extract or vitamin B_{12} for remissions if the dietary ascorbic acid is low and the administration of ascorbate has resulted in reticulocyte responses. Mueller concluded that the metabolism of ascorbic acid was altered in these subjects[93]. The administration of ascorbic acid and folic acid markedly increased the urinary excretion of folinic acid, the N^5-formyl derivative of tetrahydrofolic acid[94]. It is not possible to conclude that ascorbic acid plays a specific role in the conversion of folic to folinic acid, but it is clear that in ascorbic acid deficiency there is impairment of the functions of the folic-tetrahydrofolic system. Significant in this connection is the increased urinary excretion of p-HHP in pernicious anemia patients and in nontropical sprue[95].

S. Miscellaneous.

Mention must be made of the probability of general relationships, at least, between the ascorbic acid-dehydroascorbic acid system and other biological oxidation-reduction systems, especially the sulhydryl-disulfide systems, cysteine-cystine, and oxidized and reduced glutathione. Reference has been made to such a relationship with ferrous and ferric iron and, undoubtedly, ascorbic acid reacts similarly with copper and other metals. In one sense, the association with the folic acid-folinic acid reaction is in this category. Similarly, ascorbic acid has been related to the easily oxidized tocopherols and vitamin A.

Surprisingly, the few reports that have appeared relating ascorbic acid and ionizing radiations have been negative[96, 97]. An exception is a report in the Russian literature indicating that ascorbic acid deficiency was precipitated in guinea pigs by acute radiation injury[98].

The relation of ascorbic acid to the stress of exposure to cold has been emphasized (Section N). With reference to stresses generally, it should be emphasized that ACTH causes depletion of the ascorbic acid in the adrenal gland, but there is no clear evidence of a relationship between the vitamin and adrenal cortical functions. As noted by Kark there is no history of coexistence of scurvy and Cushing's syndrome and there is no failure of cortical secretion in scorbutic guinea pigs[99]. On the other hand, the continued administration of corticotropin has been found to produce frank scurvy in occasional individuals with prompt remission of the symptoms following administration of ascorbic acid with the hormone[100, 101].

T. Summary of Present Knowledge of Role of Ascorbic Acid in Metabolism.

Scurvy in man and in a limited number of animal species is the end result of a dietary lack of ascorbic acid. No substitutes for the ascorbic acid-dehydroascorbic acid system are known to prevent this deficiency disease. The dietary requirement of ascorbic acid is due to an inability to synthesize the compound, as is done in most animal species, because of a genetic fault which results in the absence of a single enzyme.

On the basis of studies in man, apes, and guinea pigs, it can be stated unequivocally that scorbutic animals are unable to produce connective tissue at a normal rate. The failure to synthesize collagen fibers normally is a primary defect. Whether or not the failure is absolute or relative to the need is still unsettled. Associated faulty manufacture of the matrix or ground substance of connective tissue may also be a primary defect or it may be a secondary result of the absence of collagen fibers. The inability of scorbutic animals to synthesize collagen fibers is definitely related to a breakdown in the biochemical mechanism for the hydroxylation of certain amino acids to their hydroxy-derivatives.

An impairment of connective tissue biogenesis is the probable cause of characteristic signs of scurvy such as disorganization of calcification of bones and teeth, delayed wound healing, loss of tone of walls of venules, and hemorrhage.

Apparently unrelated to the role of ascorbic acid in connective tissue formation is its participation in adjustment to cold environments. The experimental data on this question do not permit a final conclusion regarding the primary mechanism of action. Involved in some way are the medullary and cortical hormones of the adrenal glands and the hormone of the thyroid gland.

In additional activities, ascorbic acid promotes the absorption and transport of iron and supports the conversion of tryptophan to serotonin, the intermediary metabolism of phenylalanine and tyrosine, and the normal cycling of the tricarboxylic acid cycle. It may influence the production of insulin.

The effects on tryptophan and on the aromatic amino acids appear to result from involvement in the hydroxylation reaction, as in the case of proline. Part, at least, of the influence of ascorbic acid on the adrenal and thyroid hormones can also be ascribed to its role in hydroxylation reactions.

Attempts to explain the biochemical activity of ascorbic acid on the basis of its reducing power and its position in an oxidation-reduction system have been only partially successful. There is evidence, however, that an active intermediate, monodehydroascorbic acid, may serve in an electron transport mechanism which is active in the hydroxylation reaction, at least, and which may have broader functions.

There is no convincing evidence that ascorbic acid has a specific catalytic role in metabolism as a component of a coenzyme in an enzyme complex. Indeed, the relatively large dietary requirement and the size of the tissue stores, compared with other vitamins, suggest that its part in metabolism is chemical rather than catalytic. From this viewpoint, the nonspecific character of certain effects of ascorbic acid is understandable. The enigma of the involvement of a wide variety of unrelated drugs in the metabolism of ascorbic acid may also reflect the multiple nature of the vitamin's participation in important reactions in the body.

III. Ascorbic Acid Intakes and Nutriture

A. Dietary Standards for Ascorbic Acid.

Recommended dietary allowances (RDA) of ascorbic acid for infants, children, and normal adults are listed in Table 7. The recommended intakes represent the opinion of the National Research Council's Food and Nutrition Board and are "designed to maintain good nutrition in healthy persons in the United States under current conditions of living and to cover nearly all variations of requirements for nutrients in the population at large"[102]. Canadian standards seek to establish a nutritional floor beneath which maintenance of health of the people cannot be assumed. The British standard aims at maintenance of good nutrition in the average person. Accordingly, these two recommended allowances are considerably lower than those recommended for the United States.

The allowances (RDA) listed in Table 7 are based on "(a) intakes known to protect against severe or mild signs and symptoms of scurvy for individuals ranging in age from infancy to maturity; (b) the quantity supplied to infants by human milk when the mother's intake of vitamin C is characteristic of good diets; (c) human and animal intakes that maintain specific functions such as wound healing, enzyme activity, cellular proliferation, and resistance to common stresses; (d) variations in tissue concentration that result from different intakes, and related observations of the risks of tissue injury or functional disturbance; and, (e) comparative studies in nutrition, including animals that require vitamin C for protection from scurvy (guinea pigs and primates) and animals that maintain a "normal" concentration of the vitamin by tissue synthesis." These allowances have little applicability in those other parts of the world where there is restricted availability of rich food sources of ascorbic acid and where the populations may have adjusted to lower intakes of the vitamin. The data in Table 8 are based on the experience of nutrition survey teams of the Interdepartment Committee on Nutrition for National Defense and represent the relation of the observed nutritional status to intakes in other countries[103].

The interrelationship of intake, blood level, urinary recovery of a test dose, and tissue saturation is illustrated in Table 9. Needed is the additional information which would describe the nutritional status of an individual at any stated level of tissue saturation and the influence of stresses on the tissue level and on nutritional well-being.

TABLE 7

Recommended dietary allowances* of ascorbic acid.
(National Research Council's Food and Nutrition Board, 1958) (102)

	mg./day		mg./day
Men	75	Children, 7-9 years	60
Women	70	Boys, 10-12 years	75
Women, 3d trimester of pregnancy	100	13-15 "	90
Women, lactating	150	16-20	100
Infants, 1st year	30	Girls, 10-12 years	75
Children, 1-3 years	35	13-15 "	80
4-6 "	50	16-20 "	80

*Designed for the maintenance of good nutrition of healthy persons in the United States under present conditions and planned to be adequate to cover individual variations in a substantial majority of the population.

TABLE 8

Relation of daily intake and of plasma levels of ascorbic acid to nutritional status.
(Interdepartmental Committee on Nutrition for National Defense) (103)

Nutritional status	Intake mg./day	Plasma level mg./%
High	> 50	> 0.4
Acceptable	30-50	0.2-0.4
Low	10-30	0.1-0.2
Deficiency	< 10	< 0.1

TABLE 9

General relation of intake of ascorbic acid to plasma and buffy coat levels and to tissue saturation.

Tissue saturation	Urinary recovery of test dose %	Plasma level mg.%	Buffy coat level mg.%	Estimated Daily intake mg.
Saturation	60-80	>1.0	27-30	>100
Less than saturation	20-60	0.4 -1.0	15-20	40-100
One-half saturation	<10	0.2 -0.8	12-15	10-15
One-quarter saturation	< 5	0.05-0.6	5-10	5-7

Modified from O. H. Lowry, Physiol. Rev. 32, 431 (1952).

TABLE 10

Illustrative intakes of ascorbic acid in the United States*.

Study of 1804 individuals in 6 northeastern states**	Per cent with intake less than ½ RDA	Study of 446 families in Kansas and Ohio and of 9-11-year-old children in the families***	Per cent with intake less than 2/3 RDA
Men	6	Families, Kansas	3
Women	25	Ohio	4
Women, pregnant	25	Boys, Kansas	41
Boys, 13-15 years	12	Ohio	33
16-20 years	37	Girls, Kansas	35
Girls, 13-15 years	15	Ohio	31
16-20 years	11		

*Intakes relative to National Research Council's Recommended Dietary Allowances (RDA).

**Bulletin No. 319, Rhode Island Agricultural Experiment Station, University of Rhode Island, June, 1952(105).

***Bulletin No. 769, California Agricultural Experiment Station, University of California, October, 1959(106).

B. Actual Intakes of Ascorbic Acid.

Scurvy is relatively uncommon in the United States and it must be concluded that only rarely is the intake of ascorbic less than 10 mg. daily. It would be surprising if this were not the case because the average food mixture available for use by families in the United States in 1955 contained 106 mg. of ascorbic acid per person daily[92]. Nevertheless, the possibility of scorbutic diets remains because in 1955 the percentages of family diets not providing the recommended dietary allowances listed in Table 7 were as follows: Northeastern region, 17%; North Central region, 19%; Western region, 23%; and, Southern region, 37%[104]. More pertinent are the figures in Table 10 which indicate that in a significant number of cases the ascorbic acid available in the family diet in the United States is not shared equally by all individuals in the family, adolescents most frequently failing to consume their share of ascorbic acid[105, 106].

Table 11 illustrates intakes and serum ascorbic acid values in groups of elderly persons. Striking are the low average intake of a group of men living in an institution and the very great range of the serum values[107]. The mean serum value of 1.07 mg.% for the group of women masks completely the fact that individuals in the group showed levels as low as 0.07 mg.%, a level that might well be expected to result in acute scurvy if continued for too long a period.

Table 12 shows the considerable variation in intakes of ascorbic acid in military populations in other countries surveyed by the Interdepartmental Committee on Nutrition for National Defense. In no case was the average intake less than 10 mg. daily, hence scurvy was not encountered. However, these data suggest a correlation between low intakes of the vitamin and increased incidence of gum pathology[108].

Table 13 shows the results of a study of serum ascorbic acid levels in pregnant and lactating women. Despite the large number of individuals with serum values below 0.20 mg.%, there was no evident relation between the serum level and 5 conditions possibly associated with a deficiency of the vitamin, viz., gingivitis, hematologic findings, premature separation of the placenta, premature birth, and puerperal fever[109].

Table 14 is particularly pertinent to this report because it illustrates serum levels of ascorbic acid in surgical patients. Over one-third of each of the groups was considered deficient on the basis of serum and buffy coat levels less than 0.20 mg.% and 8.0 mg.%, respectively. Wound dehiscence was found more frequently in the deficient group. Crandon recommends intakes of 100 to 300 mg. of ascorbic acid daily in surgical patients[110].

Coon has made extensive studies of the relation of ascorbic acid intakes to serum and buffy coat levels in postoperative patients[111]. The control group of 61 normal persons showed a mean serum level of 0.63 mg.% with a range from 0.18 to 1.52 mg.%. Buffy coat levels in 15 normals averaged 25.0 mg.% with a range from 18 to 36.5 mg.%. The relation between serum and buffy coat levels was as follows:

Serum level	Buffy coat level
0.30 mg.% or less	21.0 mg.% or less in 95% of cases
0.30 - 0.50 mg.%	13 - 27 mg.%
over 0.50 mg.%	17 mg.% or more

TABLE 11

Serum ascorbic acid levels and dietary intakes in men and women over 50 years of age.

	No. of subjects*	Serum ascorbic acid mean mg.%	Serum ascorbic acid range mg.%	Daily intake** mean
Women	293	1.07	0.07-2.53	86
Men, Group A	232	0.83	0.11-2.28	99
Men, Group B	44	0.27	0.05-1.16	40

*All subjects lived at home except men in Group B who lived in a county home.

**Does not include ascorbic acid in vitamin supplements taken more or less regularly by 11% of the women and 18% of the men.

Revised from A. F. Morgan, H. L. Gillum, and R. I. Williams, J. Nutrition 55, 431 (1955)(107).

TABLE 12

Summary of data on ascorbic acid nutriture in military populations surveyed by Interdepartmental Committee on Nutrition for National Defense.

Nation	Number of examinations: clinical	serum ascorbate	mess surveys	Prevalence of: marginal redness or swelling of gums	bleeding or scorbutic-type gums	Ascorbic acid: in serum	in diet*
				%	%	mg.%	mg./day
Iran	1730	410	5	22	13	-	18
Pakistan	1568	409	9	25	7	-	26
Korea	1365	271	4	22	0.4	-	108
Philippines	433	96	9	13	0.2	0.47	54
Turkey	1431	277	7	33	11	0.03	15
Libya	538	142	6	45	7	0.08	20
Spain	1985	481	11	14	2	0.33	64
Peru	1315	260	7	23	3.5	0.43	73

*As calculated from standard food tables.

From I. C. Plough and E. B. Bridgforth, Public Health Reports, 75, 699 (1960)(108).

TABLE 13

Ascorbic acid levels in pregnancy and lactation.

	1st trimester	2d trimester	3d trimester	Postpartum Lactating	Postpartum Nonlactating
No. of subjects	338	1369	1960	827	637
Median serum level, mg.%	0.46	0.47	0.35	0.16	0.29
No. with serum level <0.20 mg.%	72	315	638	510	237
No. with serum level <0.40 mg.%	152	604	1096	668*	414

*Average serum level of lactating women with intake of 120 mg., or more, daily--0.30 mg.%.

Revised from M. P. Martin, E. Bridgforth, W. J. McGanity, and W. J. Darby, J. Nutrition, 62, 201 (1957)(109).

TABLE 14

Serum ascorbic acid levels in surgical patients and relation to wound healing.

	Ascorbic acid mg.% serum	buffy coat
Normals	0.69	15
Scorbutics	0.08	3.6
Surgical patients, 875	0.36	13.7
Deficient* among this group, 272	0.11	5.4
Subjected to laparotomy, 287	0.37	14.9
Deficient among this group, 115	0.11	5.2
Sufficient among this group, 172	0.52	18.9
Deficient with dehiscence, 16 or 13.3%	0.12	6.9
Sufficient with dehiscence, 3 or 1.7%	0.46	21.1

*Considered deficient if serum and buffy coat levels are less than 0.20 mg.% and 8.0 mg.%, respectively.

From J. H. Crandon, R. Lennihan, Jr., S. Mikal, and A. E. Reif, Ann. N.Y. Acad. Sci., 92, 246 (1961)(110).

An average daily intake of 100 mg. of ascorbic acid could be expected to result in a mean buffy coat response which was maximal between the range of 25 - 30 mg.%. However, the variability was too great to permit the estimation of the 95% confidence limit for a buffy coat value of 20 mg.%.

On the basis of group averages, the following relation between intake of ascorbic acid and whole blood level in preoperative subjects was found:

Intake mg. per day	Whole blood level mg.%
50	less than 0.40
75	0.35 - 0.60
100	0.50 - 0.80
125 - 150	0.75 - 1.00

Here, also, individual variation within groups appeared to be of such significance that the problem was examined in considerable detail. A group of 130 patients requiring major operations was followed during the postoperative period while on fixed intakes of ascorbic acid varying between 0 and 300 mg. per day. Serial determinations of ascorbic acid content in whole blood were made in all patients and simultaneous assays of levels in buffy coat were performed in 111. From regression analysis of changes in blood levels in these patients during the period of fixed intake, it appeared that 200 mg. of ascorbic acid per day are required to elevate or maintain these levels at a range compatible with "near-saturation of tissues" (blood level of 0.4 mg.% or above) in at least 95% of a similar population sample. However, a blood level of 0.2 mg.% or above, which is probably sufficient to prevent defects in wound healing, can be achieved with 95% confidence with an intake of approximately 75 mg. of ascorbic acid per day.

Comparison of regression equations for mean response in blood ascorbic acid in this group of postoperative patients with data reported by Lowry et al. for young healthy adults revealed little difference between dose required to sustain comparable mean blood levels[11]. Individual variability in apparent requirements, however, was very large in both groups. It was concluded that more information is necessary concerning differences in requirements for ascorbic acid of normal adults before an accurate assessment of any increased needs of the surgical patient can be made. Furthermore, the data indicate that patients subjected to operative trauma of the usual magnitude did not require an intake of ascorbic acid greatly exceeding that probably required by normal healthy adults.

Russell has summarized the observations on periodontal disease and ascorbic acid nutriture that have been made on over 21,000 persons in 8 geographical regions of the world under the auspices of the Interdepartmental Committee on Nutrition for National Defense[112]. Most of the variance in periodontal disease was associated with faulty oral hygiene and with age, although populations with considerable periodontal disease tended to be deficient in vitamin A. No association between periodontal disease and ascorbic acid intake could be demonstrated.

According to Army Regulations (AR40-564) the garrison or field-type ration must

provide 75 mg. of ascorbic acid per man per day. Whole blood ascorbic acid determinations on 702 enlisted men in 4 military installations showed an average value of 0.83 mg.% (S.D. ± 0.33)[113]. The distribution of individual values showed 7 men with levels between 0.10 and 0.20 mg.%, 60 between 0.20 and 0.40 mg.%, and the remainder over 0.40 mg.%. These data indicate either that 10% of the men failed to ingest the foodstuffs providing the recommended quantity of the vitamin or that in 10% of the total group the ingestion of 75 mg. of ascorbic acid daily failed to result in expected blood levels. At the time of procurement operational rations provide 115 to 150 mg. of ascorbic acid per individual per day. Blood levels in troops consuming these rations are not available.

In emphasizing the desirability of intakes of ascorbic acid that maintain specific levels in the blood, 0.40 mg.% for example, it should be noted that analyses of plasma (or serum) and of whole blood yield similar values when expressed as mg.% of the respective fluids. If buffy coat levels are high, whole blood values may be slightly larger than plasma values but the difference is generally without significance. The difference may become significant in depleted individuals whose white cells still retain some ascorbic acid after its virtual disappearance from the plasma[114]. Determinations of ascorbic acid in whole blood by oxidation-reduction procedures proved unreliable because of the interference of oxyhemoglobin. This difficulty was overcome by conversion of oxyhemoglobin to carbon monoxide hemoglobin[114] and by the introduction of the Roe and Keuther method in which ascorbic acid is oxidized to dehydroascorbic acid and coupled with 2,4-dinitrophenylhydrazine[115].

C. Summary of Knowledge of Human Requirements of Ascorbic Acid.

Scurvy in man does not occur if the diet provides 10 to 20 mg. of ascorbic acid daily. The rate of metabolic utilization in the normal male adult is approximately 0.21 mg. per day per kilogram of fat-free body weight. This is the equivalent of 10 to 20 mg. daily.

Tissues of most animals in which synthesis of ascorbic acid occurs and in which dependence on a dietary source is unnecessary contain amounts of the vitamin that approach saturation levels. In a group of normal adult men, near-saturation levels can be obtained on a mean daily intake of 100 mg. but many individuals in the group would be less than saturated. An intake of 10 to 20 mg. daily would permit only partial saturation, the corresponding average plasma levels and percentages of saturation of tissues being estimated as 0.20 mg.% and 30%, respectively. For 50% saturation the estimated intake is 40 to 60 mg. daily and the average plasma level 0.40 mg.%. However, to assure an average blood level of 0.20 mg.% with 95% confidence an average intake of 75 mg. daily would be required and to assure an average blood level of 0.4 mg.% with 95% confidence an average intake of 200 mg. would be necessary.

The important question is whether or not there is a significant improvement in physical and mental performance, in endurance in the face of severe stress, and in the rate of recovery from injury, disease, or other stress if an individual consumes amounts of ascorbic acid greater than the minimum requirement for the prevention of the more easily recognized evidences of scurvy. In other words, is there an advantage in maintaining a higher degree of tissue saturation than is possible on an intake of 10 to 20 mg. daily, an advantage other than that of having a factor of safety that may provide insurance against unforeseen increased needs of the vitamin? On the other hand, can it be taken for granted that higher degrees of saturation of tissues with ascorbic acid are entirely without detriment?

Although the average level of tissue saturation with ascorbic acid correlates well with average intakes in circumstances which can be considered normal, this is not necessarily the case under abnormal conditions. Certainly such a correlation cannot be taken for granted in the case of individuals in either normal or abnormal circumstances. For these reasons, it is imperative to distinguish clearly between preventive or therapeutic measures that are taken on the basis of group averages and those taken on the basis of individual analytical data in which the plasma (or whole blood) and the buffy coat (leucocytes and platelets) levels are used as indices of intakes and tissue storage, respectively. It is emphasized that much more information is needed before the relationship of these values to the actual utilization of the vitamin is understood. In spite of the paucity of data on these many variables, certain tentative conclusions are possible and are discussed briefly under separate headings below. For the purposes of this report, high tissue saturation is defined as a level appreciably above 50%.

High tissue saturation under normal conditions.

Even presupposing agreement on a definition of what is normal, the question of an undisputed advantage of high tissue saturation to the healthy adult male in a normal environment is difficult to answer in the affirmative because of the absence of conclusive evidence that a regimen which, for example, maintains the level of serum ascorbate near 0.20 mg.% and the percentage of tissue saturation near 30% is the direct cause of any deleterious effect. The findings in Section III-B above make it plain that

large numbers of individuals in the United States are in this category and it is not possible to prove that persons in this group exhibit signs of deficiency that are attributable only to an inadequate supply of ascorbic acid. There is even uncertainty regarding the etiology of periodontal changes which are commonly believed to be correlated with low intakes of the vitamin. There is presumptive evidence in favor of a higher intake of ascorbic acid but this point can only be established by new and better methods of recognizing borderline intakes of the vitamin.

Crandon has demonstrated that many months are required for the appearance of acute signs of scurvy if the initial concentration of ascorbic acid in the tissues is close to saturation of the tissues, as indicated by a buffy coat value of 28 mg.%. Presumably, the onset of scurvy would be hastened in the case of individuals ingesting an ascorbic acid-free diet after prior subsistence on a "normal" diet that permits only 25 instead of 75% saturation of the tissues.

High tissue saturation in conditions of stress.

In the case of healthy adults exposed to various stresses and in the case of adults who are injured or ill, the question of an advantage associated with higher degree of saturation of the tissues is complicated by a number of possible effects concerning which insufficient knowledge is available. These include (a) the extent of unusual losses in the urine and of significant losses due to perspiration, hemorrhage, or serous exudates as a result of a stress; (b) alteration of metabolism of ascorbate due to drugs, including those used in treatment; (c) alteration of utilization of ascorbate arising from hormonal and other humoral factors; (d) the specific effect of the stress itself, whether it be exposure to extremes of temperature, deprivation of food, or any other condition imposing demands on physiological structures or mechanisms; and (e) the effect of multiple stresses. In view of these complex and, for the most part, uninvestigated factors, no general statement can be made other than that no circumstances are known in which less than normal intakes of ascorbic acid are desirable. The problem is whether or not the evidence justifies intakes of the vitamin greater than the so-called normal amounts.

Trauma.

A beneficial effect of high levels of tissue saturation with ascorbic acid is believed to have been established in the case of healing processes, especially in skin grafts, in patients with severe and extensive burns. The benefit has been demonstrated also in severe trauma but is less evident in moderate injuries and may be absent in the case of minor operations or wounds. It can be assumed from the findings in guinea pigs that the plasma and tissue supply of ascorbic acid must be sufficient to maintain the required rate of formation of new connective tissue plus the support of other ascorbate-using processes. In severe trauma it appears difficult to provide the necessary concentration in the blood except by the administration of relatively large amounts of the vitamin. Whether or not this is the result of accelerated rates of utilization, catabolism (destruction), or loss in exudates is not known. There is an increase in the level of tissue ascorbic acid in the healing area.

No specific statement can be made regarding the quantitative requirement of ascorbic acid in severe trauma. It would appear reasonable, however, to insure an intake that would maintain a plasma level of 0.40 mg.% regardless of whether this required 100 or 1000 mg., or more, per day. It is important to recall that an average intake of 200 mg. is necessary to insure this blood level in 95% of a group of normal men and of postoperative patients. In the absence of laboratory analyses, only a larger amount, possibly 300 mg. or more, would make the desired 0.40 mg.% level possible in the case of the other 5%. In severe trauma much larger intakes would probably be required to insure this blood level.

Infection.

Synergism is the usual finding with respect to ascorbic acid, susceptibility to infection, and severity of the infectious process. Pertinent experimental studies have involved scorbutic guinea pigs and monkeys and the vulnerability of the animal, with a borderline deficiency, to both scurvy and infection is clear. Advantages of high tissue saturation have been reported in animals but satisfactory proof in man of significant benefits of intakes of ascorbic acid larger than the 75 mg. per day of the recommended dietary allowances (RDA) has not appeared.

Urinary oxalate.

Ascorbic acid and the amino acid, glycine, are the principal precursors of urinary oxalic acid. Insofar as the fraction originating from ascorbate is concerned, the present evidence indicates that it is produced and excreted in proportion to the metabolic utilization of the vitamin. Excess dietary ascorbic acid is excreted as such. There is no evidence that oxalate calculi can be ascribed to the ingestion of ascorbic acid, even in large amounts.

Exposure to cold.

A large body of evidence based on animal studies, including findings in animals that synthesize ascorbic acid, suggests that high tissue saturation should be advantageous in the face of exposure to severe cold. Limited investigations on this problem do not support a similar conclusion in man although, admittedly, the picture is not clear. A beneficial effect was demonstrated in the case of men subsisting in a cold environment on a diet markedly deficient in calories. Noteworthy, also, was the observation that plasma ascorbate was significantly lower in men exposed to cold even though the diet was rich in calories and ascorbate. In view of the uncertainty regarding the usefulness of a high tissue level of ascorbate in men exposed to severe cold, the provision of supplementary ascorbate appears a reasonable action pending the determination of the effect of cold stress on the rate of utilization of the vitamin.

Drugs.

The surprising effects of various pharmacologically active compounds on the metabolism and excretion of ascorbic acid are of such magnitude in animals as to emphasize the importance of awareness of the possibilities in men exposed to chemical agents which may be either occupational hazards or medicinals used in treatment. It should be recognized not only that the utilization and requirement of ascorbic acid may be affected but also that the degree of activity of drugs may be influenced favorably or adversely by high tissue levels of ascorbate.

Multiple stresses.

Combinations of stresses would be expected to increase the rate of utilization of ascorbic acid but no such conclusion can be drawn on the basis of existing evidence except in the case of trauma and, possibly, of exposure to cold.

Individual variations.

The extreme differences in ascorbic acid nutriture observed in individuals has been emphasized repeatedly in this report. A better understanding of the factors associated

with individual variability might yield clues on methods of coping with this problem and might also provide new data on the functions of ascorbic acid in metabolism. This is a problem meriting vigorous study.

Preventive saturation of tissues.

The usefulness of intakes of ascorbic acid greater than 75 mg. daily in order to increase the level of the vitamin in the tissues has yet to be proved in the case of normal men not exposed to abnormal situations. Care is required, however, to insure an intake of 75 mg. on an individual basis.

In the case of military personnel assigned to hazardous environmental and operational situations, controlled investigations on the optimum level of intake have not been performed. Pending such studies, intakes that would give assurance of a blood level of 0.40 mg.% with a confidence level of 95% appear reasonable. Amounts larger than this, i.e., more than 200 mg. daily, are not believed necessary because near-saturation would be achieved by the 200 mg. intake. Furthermore, tissue depletion resulting from an increased utilization, catabolism, or excretion of ascorbate due to stress can be repaired quickly by the administration of ascorbic acid. It is clear that 1000 mg., or more, daily can be provided safely.

Method of administration of ascorbic acid.

Ascorbic acid in large amounts, 1000 mg. or more per day, can be administered effectively orally or parenterally. In view of the absence of demonstrated detrimental effects as results of an excess of ascorbate, there is no necessity for use of special preparations to insure activity over longer periods. It is reasonable to administer ascorbate in divided doses as a means of avoiding excessive elevation of the plasma level above the renal threshold. For this reason the oral route is to be preferred. Topical application on exposed tissues after burns is effective but an advantage of this method has not been demonstrated.

Adaptation.

The possibility that the ascorbic acid nutriture of individuals is influenced by adaptive mechanisms that are adjusted to previous and current levels of intake of the vitamin should not be ignored. This could be an explanation of the virtual absence of signs of scurvy in some populations in which the intake and the plasma level of ascorbate have been low for long periods and in which the general level of nutrition appears better than should be expected on the basis of the available foodstuffs. Abundant evidence now supports the hypothesis that activities of tissue enzymes vary in proportion to the levels of their substrates. It is important to know whether or not the "luxus" consumption of ascorbic acid favors an accelerated rate of catabolism, as well as of excretion, of the compound with a continuation of either or both at an elevated rate for a period after cessation of the "luxus" intake.

Interrelationships with other nutrients.

The influence of ascorbic acid in iron and folic acid metabolism and in the metabolism of several amino acids has been demonstrated. The involvement of ascorbate in certain of the reactions of intermediary metabolism in which other vitamins and trace elements participate suggest that it may also influence the activities of thiamine, riboflavin, pantothenic acid, pyridoxine, and copper. A relationship to vitamins A and E is clearly possible, even if only on the basis of oxidation-reduction systems. The significance of these relationships on the nutritional status of poorly nourished populations has not been determined.

IV. Current and Proposed Research Applicable to Military Needs

A. Evaluation of Research Supported by Army.

Appendix C lists Army-supported projects, with summaries of the proposed research, as reported to Science Information Exchange since 1959. These include the following aspects of ascorbic acid nutriture:

Study of nutrients, including ascorbic acid, in young adults subjected to moderate stresses that are a part of student life on a university campus.

Determination of usefulness of roentgenograms of mandible of guinea pig as a measure of antiscorbutic activity of diets.

Study of sodium balance, nitrogen utilization, fat absorption, and ascorbic acid needs in children after trauma, including burns.

Study of nutritional status of military populations, including measurement of plasma ascorbic acid.

Determination of role of glucuronic acid and glucuronolactone in human ascorbic acid deficiency.

Study of treatment of induced oral lesions in guinea pigs, including use of ascorbic acid.

Study of biochemical and metabolic changes associated with traumatic and irradiation injury in man and animals, with special emphasis on role of ascorbic in wound healing after severe burns. (Two projects).

These projects represent reasonable approaches to certain facets of the ascorbic acid problem but fall far short of answering the questions that are the basis of this report. On the other hand, the list includes projects in which there are findings of great importance. The study of the metabolism of glucuronates has led to measurements of rates of utilization of ascorbic acid in man. The extension of this work to include measurements during various types of stress should give direct answers to the question of the effect of stress on ascorbate requirements. The studies on wound healing are also of unusual significance because of the clear-cut demonstration of the occurrence of "biochemical" scurvy during severe trauma. The extension of this work to germ-free animals is also important for the understanding of the influence of microorgamisms on ascorbic acid nutriture. None of these projects deals directly with the fundamental basis of action of ascorbic acid or with the role of ascorbate in cold exposure, with the interrelationship of the vitamin and drugs, or with the clarification of the problem of periodontal disease as an index of ascorbic acid nutriture.

B. Evaluation of Research Supported by Other Agencies.

The Science Information Exchange lists 45 projects concerned with ascorbic acid since 1959. Of these, 32 were supported by the National Institutes of Health, 9 by the Veterans Administration, and 4 by three nongovernmental agencies. It is recognized that a considerable proportion of the current studies described in Section II is not included among the projects listed by the Science Information Exchange.

An approximate classification of these investigations shows the following distribution: collagen formation, 11; intermediary metabolism, 8; dental nutrition, 7; trauma, 5; nutrient interrelationships, 4; other stresses, 4; miscellaneous, 6. None dealt with the role of ascorbic acid in cold stress and only one represented a basic investigation of the antiscorbutic function of the vitamin. Despite the value of many of the projects, the investigations as a group do not measure up to the importance of the findings that remain to be disclosed.

The apparent lack of interest among many investigators in solving the enigma of the real function of ascorbic acid as an essential nutrient is puzzling. Possibly some have assumed that the problem has been solved by the experiments linking the biogenesis of hydroxyproline and collagen. But the details of these processes are only sketchily known and the extension of the hydroxylation reaction to other compounds is in a similar state of incompleteness. Also, the confusing, partial replacement of ascorbic activities by an assortment of other substances may have discouraged some investigators. However this may be, the fact remains that without ascorbic acid man cannot live. This is a challenge which should be met by vigorous and skillful experimentation.

C. Problems Meriting Further Investigation.

Problems meriting further study are listed in the form of questions in Appendix D.

V. Conclusions

A. General Summary.

1. Research of importance in military life and operations.

Current research on ascorbic acid that is applicable to military needs has been discussed in Section II. The military importance of these studies is indicated by the fact that the formation of connective tissue is indispensable for wound healing and that research on the biogenesis of collagen of connective tissue is, in fact, research on a basic aspect of the function and mechanism of action of ascorbic acid. The hydroxylation reaction, on which the synthesis of collagen's hydroxyproline depends, may be the metabolic reaction that underlies much of the vitamin's activity in the body. Admittedly, there is no proof that the favorable influence of ascorbic acid on the synthesis of hydroxyproline represents the sole manner in which the vitamin may function. Nor has it been demonstrated that this one particular hydroxylation reaction is involved also in the metabolism of para-hydroxyphenylpyruvic acid, of tryptophan, of aromatic amino acids in the synthesis of adrenal medullary hormones, nor of steroids in the formation of the hydroxysteroid derivatives secreted by the adrenal cortex. Nor is it certain, or even likely, that the favorable influence of ascorbic acid on the absorption, transport, and utilization of iron, on the enzymes of the tricarboxylic acid cycle; on dentinogenesis by odontoblasts; on mucopolysaccharides of "ground" substance; and on the folic acid-tetrahydrofolic acid system is associated with the hydroxylation mechanism. But even these processes might be dependent on an electron transport reaction in which monodehydroascorbic acid may be a labile, active component.

On the other hand, it is quite possible that the ascorbic acid-dehydroascorbic system may influence favorably those reactions which are dependent on an oxidation-reduction mechanism of this type. If so, there is urgent need of research that will clarify the distinction between ascorbate-dependent reactions which may be considered primarily antiscorbutic in character and those reactions which are optimal in a physico-chemical environment equivalent to that provided by ascorbic acid and its oxidation product. Existence of reactions of this latter type, that appear to be ascorbate-dependent, would explain in part, at least, the nonspecificity which has been demonstrated in the case of isomers, such as D-ascorbic acid, that possess partial activity.

Methods are now available for the measurement of the rate of utilization of ascorbic acid and the application of the procedure in men who are undergoing stress of various types is of the greatest importance. Particularly urgent is the need of information on the rate of utilization during severe trauma and during exposure to cold. The fate of the large amount of administered ascorbic acid which is presently unaccounted for in patients with extensive burns or wounds must be ascertained. Needed also is an answer to the question of possible adaptation to low intakes of ascorbic acid. Much more understanding of the interaction of hormones and ascorbic acid is essential.

The whole problem of the role of ascorbic acid in metabolism will remain confused until there is complete understanding of the basis of the stimulation of synthesis and breakdown of the vitamin by a variety of chemical agents in animals that are not dependent on a dietary source. Likewise, an understanding is essential of the influence of certain drugs on ascorbate-dependent reactions in animals that are

dependent on exogenous ascorbic acid. The importance of these questions for man is unknown but the implication is obvious and cannot be ignored.

An explanation of the individual variability of men with respect to ascorbic acid needs is required, as is also a simpler and more informative measure of the ascorbic acid nutriture.

2. Categories and adequacy of intakes of ascorbic acid.

Stress, whether it is in the form of incapacitating physical exertion, illness, trauma, exposure to extremes of environmental temperature, or any other markedly abnormal circumstance, is a constant hazard facing military personnel. Preventive measures that will assure the maximum possible protection against losses of operational efficiency are a necessity. Assurance of an adequate daily intake of ascorbic acid is one such preventive measure.

Three levels of intake of ascorbic acid for prophylaxis are referred to in this report, as follows:

a. The minimum requirement for the prevention of scurvy is 10 to 20 mg. daily. This level of intake will result in partial saturation only, estimated as 30 to 40%. Corresponding plasma ascorbate levels will average 0.20 mg.% or less.

An intake of ascorbic acid greater than the minimum requirement is recommended under all circumstances.

b. The recommended allowance (RDA) of the National Research Council for normal male adults is 75 mg. daily and this is also the amount authorized by AR40-564. This level of intake will result in a higher degree of tissue saturation, estimated as 50 to 90%. Corresponding plasma ascorbate levels will range from 0.10 to 1.00 mg.% with 95% confidence that the level will be above 0.20 mg.%.

Emphasis is placed in the report on the desirability and safety of near-saturation of the tissues with ascorbic acid. This is achieved in some but not in all male adults by the ingestion of 75 mg. daily. Nevertheless, this amount is sufficient to prevent scurvy and to give protection in most individuals against the moderate stresses that have been studied.

c. The supplemented allowance is one which is appreciably in excess of 75 mg. daily and may range from 200 to as much as 1000 mg. daily. Tissue saturation is the objective. Corresponding plasma ascorbate levels will range from 0.30 to 1.80 mg.%. An intake of 200 mg. daily will assure a plasma level of 0.40 mg.% or more with 95% confidence. This plasma level is believed desirable for all military personnel selected for particularly hazardous environmental and operational assignments.

Severe trauma, especially trauma resulting from extensive burns, is a stress which imposes an additional demand for ascorbic acid. The extent of the demand can probably be estimated by the determination of plasma ascorbic acid. The administration of ascorbate should be sufficient in amount to maintain the plasma level above 0.40 mg.%, at least. The ascorbate can be provided orally or parenterally and is more efficiently utilized if administered in divided doses. Patients undergoing elective surgery, in general, require a daily intake of 200 mg. to insure the 0.40 mg.% level with 95% confidence. Larger intakes are necessary for 100% confidence and, also, in the case of very severely traumatized patients. For the latter, an intake of 1000 mg. daily may be required.

Exposure to severe cold is tentatively judged to constitute a stress that may be offset in part by supplementary ascorbic acid. The additional ascorbate should be sufficient in amount to maintain the plasma level of ascorbic acid above 0.40 mg.%, at least.

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